Acetate Metabolism in a pta Mutant of Escherichia coli W3110: Importance of Maintaining Acetyl Coenzyme A Flux for Growth and Survival

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In order to study the physiological role of acetate metabolism in Escherichia coli, the growth characteristics of an E. coli W3100 pta mutant defective in phosphotransacetylase, the first enzyme of the acetate pathway, were investigated. The pta mutant grown on glucose minimal medium excreted unusual by-products such as pyruvate, n-lactate, and l-glutamate instead of acetate. In an analysis of the sequential consumption of amino acids by the pta mutant growing in tryptone broth (TB), a brief lag between the consumption of amino acids normally consumed was observed, but no such lag occurred for the wild-type strain. The pta mutant was found to grow slowly on glucose, TB, or pyruvate, but it grew normally on glycerol or succinate. The growth rate and starvation survival of the pta mutant were restored by the introduction of poly-β-hydroxybutyrate (PHB) synthesis genes (phbCAB) from Alcaligenes eutrophus, indicating that the growth defect of the pta mutant was due to a perturbation of acetyl coenzyme A (CoA) flux. By the stoichiometric analysis of the metabolic fluxes of the central metabolism, it was found that the amount of pyruvate generated from glucose transport by the phosphoenolpyruvate-dependent phosphotransferase system (PTS) exceeded the required amount of precursor metabolites downstream of pyruvate for biomass synthesis. These results suggest that E. coli excretes acetate due to the pyruvate flux from PTS and that any method which alleviates the oversupply of acetyl CoA would restore normal growth to the pta mutant.

Escherichia coli excretes acetate as a major by-product of its aerobic metabolism (3). Acetate is produced from acetyl coenzyme A (CoA) via acetyl phosphate by the Pta-AckA (Pta, phosphotransacetylase; AckA, acetate kinase) pathway, which is reversible and constitutively expressed (6). The excreted acetate is reused via acetyl CoA synthetase (ACS) or the Pta-AckA pathway after the depletion of favored carbon sources such as glucose. ACS or the Pta-AckA pathway, showing higher or lower affinities for the acetate, respectively, activates acetate to acetyl CoA (24).

Aerobic acetate production depends on the growth rate. When the growth was limited by restricting the feeding rate of the carbon source, acetate excretion diminished, and, under a critical growth rate (μc), was stopped in chemostat cultures of E. coli and Aerobacter aerogenes (13, 36, 42). The specific oxygen uptake rates increased linearly with increased growth rate under μc in both strains (36, 42). At growth rates higher than μc, specific oxygen uptake rates did not increase further and acetate production started in E. coli (36). Similar findings for batch cultures with various carbon sources that supported different growth rates were reported (3). Based on the effect of the relationship between acetate production and specific oxygen uptake rate upon growth rates and the fact that acetate biosynthesis is accompanied by equimolar substrate-level ATP generation, acetate excretion is regarded as an overflow metabolism, which provides additional energy when the respiration capacity is saturated (3).

In the context of metabolic flux analysis, aerobic acetate production has been understood as an effect caused by the imbalance between the uptake of the substrate and the demand for biosynthesis and energy production (18). From stoichiometric flux analysis of the central metabolic pathways in E. coli, Holms proposed that the ratio of flux to energy supply does not limit growth and that consequently acetate excretion functions as a safety valve to balance excessive inputs to the fluxes of precursor metabolites for biomass synthesis (19). The fact that E. coli grown on glycerol or fructose does not excrete acetate was explained to be an effect of the restricted uptake of substrates. The maximal uptake rate of these substrates was not supposed to reach the threshold required to trigger acetate excretion (19). Though the analysis adequately demonstrated the existence of an imbalance between substrate uptake and the demands for anabolism, the exact cause of such an imbalance has not been explained.

A possible role of acetate metabolism is for the production of acetyl phosphate, which was recently found to be a phosphate donor for many signal transduction response regulators including CheY, PhoB, NR1, and OmpR (14, 27, 28, 33). It has been shown that the intracellular level of acetyl phosphate varies over a wide range, depending on the presence of a pta or ackA mutation, the phase of growth, and temperature (27, 35). Moreover, the pta mutant has been reported to be impaired in its ability to survive during glucose starvation, while the ability of the ackA mutant to survive remained the same as that of the parent strain (30). Therefore, providing an appropriate level of acetyl phosphate may be an important function of the acetate pathway in E. coli.

In this work, the growth phenotypes of a pta mutant were

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investigated to understand the physiological roles of acetate metabolism. Several unexpected growth characteristics, such as a large decrease of the growth rate and the excretion of pyruvate and D-lactate, were found during previous investigations of pta mutants (12, 13, 21). Because the altered acetate metabolism must cause severe metabolic flux reorientation, which should result in the accumulation of by-products, the profiles of by-product accumulation, the consumption pattern of amino acids in the complex medium, and the growth rates in cultures with a variety of carbon sources were examined in greater detail. Metabolic flux analysis, based on the calculated amounts of precursor metabolites required for the synthesis of biomass, revealed that pyruvate is always excessively generated because of the characteristics of the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS). Therefore, it is proposed that acetate production in E. coli is an unavoidable consequence of substrate transportation via PTS and that the growth defect of the pta mutant is due to the unbalanced flux of acetyl-CoA. Because the introduction of phbCAB genes redirecting acetyl-CoA flux to poly-β-hydroxybutyrate (PHB) was found to improve the growth of the pta mutant, any method of alleviating the perturbed acetyl-CoA flux would be expected to restore the normal growth of the pta mutant.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. To construct mutant strains, a pta-1::Tn10lacZ fusion was introduced into W3110 or RR1 by P1 transduction with lysates of CP993. Plasmid pSK2665 was derived from pKT230 with the 5.2-kb BamHI-EcoRI fragment consisting of phbCAB genes from A. eutrophus that provides PHB levels were determined by gas chromatography (Hewlett-Packard) with benzoi acid as an internal standard (5).

### Table 1. Bacterial strains and plasmids used

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W3110</td>
<td>F′ [pMW22266 (M9)]</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>RR1</td>
<td>supE44 hsdS2F(rB mB−) ara-14 proA2 lacY1 galK2 rpsL20 syl-5 multidrug resistance</td>
<td></td>
</tr>
<tr>
<td>CP993</td>
<td>OW1 [pBH50 (pBH50)]</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>JP202</td>
<td>RR1 [pta-1::Tn10lacZ]</td>
<td>This work</td>
</tr>
<tr>
<td>JP231</td>
<td>W3110L [pta-1::Tn10lacZ]</td>
<td>This work</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSK2665</td>
<td>phbCAB genes from A. eutrophus</td>
<td>A. Steinbüchel (34)</td>
</tr>
<tr>
<td>pKT230</td>
<td>pK230 with the 5.2-kb BamHI-EcoRI fragment bearing phbCAB genes from pSK2665</td>
<td>This work</td>
</tr>
</tbody>
</table>

**RESULTS**

The *E. coli* W3110 pta mutant accumulates pyruvate, D-lactate, and L-glutamate instead of acetate. The growth phenotypes of an *E. coli* W3100 pta mutant were compared with those of wild-type strain W3100 to study the physiological changes due to the pta mutation. In glucose minimal medium, the pta mutant JP231 grew more slowly than its parental strain, W3110. The maximum specific growth rates were 0.49 and 0.60 h⁻¹ for JP231 and W3110, respectively (Fig. 1). While the biomass yields of both strains on glucose were the same, 0.077 g (dry weight)·mM⁻¹, the specific glucose consumption rate of the pta mutant, 6.06 mM·(g [dry weight]·h)⁻¹, differed from that of wild type, 7.87 mM·(g [dry weight]·h)⁻¹. Introduction of a pta mutation did not completely eliminate acetate production during the growth phase. The acetate yield of the pta mutant on glucose, 0.11 mM·(g [dry weight]·h)⁻¹, was reduced to one-fourth that of the wild type, 0.45 mM·(g [dry weight]·h)⁻¹. Pyruvate oxidase (PoxB) presents a possible route for the generation of acetate in the pta mutant, because acetyl phosphate may be converted to acetate and inorganic phosphate through the action of either AckA or nonenzymatic degradation (6). However, a further mutation in the poxB gene did not result in the complete elimination of acetate (40). As previously reported (12, 21), pyruvate and D-lactate were excreted during the exponential growth of JP231, while in its parental strain (W3110) the excretion of pyruvate and D-lactate was quantitatively insignificant. Once glucose was exhausted, the pta mutant reused the excreted acetate mainly through ACS (24), pyruvate via pyruvate dehydrogenase (16) or pyruvate oxidase (8), and D-lactate via the NAD-independent, membrane-bound D-lactate dehydrogenase (D-LDH) (23).

Because metabolic perturbation was demonstrated by the secretion of byproducts, the possibility of acetyl-CoA being redirected in the tricarboxylic acid (TCA) cycle was examined. Acetyl-CoA incorporated into the TCA cycle may be oxidized completely to CO₂ or excreted as by-products such as intermediates of the TCA cycle or amino acids formed from such intermediates. No significant amounts of organic acids synthesized from intermediates of the TCA cycle were detected by NMR analysis. However, amino acid analysis showed that...
JP231 grown in glucose minimal medium accumulated glutamate, up to 0.33 mM from 12 mM glucose, while the amount produced by W3110 was insignificant (Fig. 2). No amino acids other than glutamate were detected. The sum of fluxes to pyruvate, D-lactate, and glutamate accounted for 96% of the acetate flux reduction in the pta mutant.

The pta mutant shows a different consumption profile of amino acids. The growth defect of the pta mutant on glucose and the accumulation of glutamate led us to check whether the consumption profiles of amino acids by the JP231 mutant, on TB containing M9 salts, differed from those of W3110. It is worth pointing out that the growth defect was more pronounced on TB containing M9 salts than on glucose minimal medium. On TB, the growth rate of JP231 was only 45% that of the wild-type strain, whereas it reached 78% of the control on glucose. Since amino acids serve as the primary carbon source, the levels of sequential consumption of amino acids by both W3110 and JP231 growing on TB containing M9 salts were analyzed (Fig. 3). The consumption profiles of amino acids in the wild-type strain were consistent with those from a previous report (34). During the early exponential growth phase, L-serine was consumed exclusively; this was followed by the consumption of L-aspartate (Fig. 3a). In this phase, acetate accumulated and was later reused when consumption moved to L-glutamate, L-threonine, L-alanine, and glycine. In the stationary phase, L-arginine was the only amino acid to be consumed. Glycine was accumulated when the cells were consuming L-serine, L-threonine, and L-alanine, indicating an imbalance between the assimilation of such amino acids and their related catabolic pathways.

The pta mutant exhibited a different consumption pattern (Fig. 3b). Although its order of amino acid consumption was the same as that of W3110, there was a lag between the initial consumption of L-serine and other amino acids. After a brief lag, L-aspartate, L-threonine, L-alanine, glycine, and L-glutamate were assimilated. This retardation in the assimilation of amino acids is consistent with a previous report concerning the nuo mutant (34). Glycine accumulation was not observed, which reflected a decrease in the assimilation rate of such amino acids. As noted in the previous section, the concentration of L-glutamate increased before it was consumed. The accumulation level of acetate by the pta mutant was lower in TB containing M9 salts than in glucose minimal medium, which indicated that the assimilation of amino acids as the carbon source results in a lower flux to acetyl CoA than does that of glucose.

The pta mutant grows slowly on glucose, TB, or pyruvate but normally on glycerol or succinate. Maximum growth rates of JP231 on various carbon sources were compared with those of W3110, because the pta mutant had shown different growth defects in glucose and TB. JP231 grew more slowly than W3110 not only in glucose and TB containing M9 salts but also on fructose and pyruvate (Table 2) (17). The specific growth rates of the pta mutant on fructose and pyruvate were 75 and 45%, respectively, of those of the wild-type strain. However, on glycerol or succinate, the growth rates of the pta mutant were comparable to those of the wild type. Metabolism of substrates on which the pta mutant showed the growth defect is directly linked to pyruvate either by PTS (glucose and fructose), catabolism of amino acids (TB), or by pyruvate itself. Because PTS using PEP as the phosphate donor links the uptake of glucose or fructose with the stoichiometric coproduction of pyruvate, oversupplied pyruvate should be excreted as acetyl CoA. Therefore, the fluxes of pyruvate and consequently acetyl CoA

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**FIG. 1.** Growth and by-product excretion profiles of W3110 (a) and JP231 (b). Strains were grown in an aerated fermentor containing M9 minimal medium supplemented with 2 g of glucose liter$^{-1}$. Symbols: ○, dry cell weight; □, glucose; △, acetate; ●, pyruvate; ▼, D-lactate.

**FIG. 2.** Glutamate accumulation in a pta mutant grown on M9 glucose minimal medium. Profiles of growth (a) and glutamate accumulation (b) of W3110 (open symbols) and JP231 (solid symbols) are represented.
in the pta mutant grown on PTS carbon sources would be expected to be severely perturbed, as is manifested by the excretion of pyruvate, D-lactate, and L-glutamate. In addition to the excretion of unusual by-products, the perturbation of acetyl CoA and possibly pyruvate fluxes in the pta mutant, in some way, causes the decrease in the growth rate.

Defective growth of the pta mutant is recovered by phbCAB introduction. In an attempt to check whether acetyl CoA flux is responsible for the decreased growth rate of the pta mutant, PHB synthesis genes (phbCAB) from A. eutrophus (38) were introduced into W3110 and JP231. Initially, the effects of introducing PHB synthesis pathway genes upon the distribution of acetyl CoA flux were examined by analyzing the by-products. In the wild-type strain, the introduction of phbCAB genes had no effect on the by-product pattern, in that acetate was excreted as the only product in M9 medium containing glucose (Fig. 4a). In contrast, the introduction of phbCAB genes repressed the excretion of the by-products, the characteristic of the pta mutant: excretion of pyruvate and D-lactate was diminished (Fig. 1b and 4b), and the maximum level of glutamate was lowered to one-sixth that associated with the pta mutant (data not shown). Moreover, the maximum level of acetate was also reduced to three-fourths that of the control mutant strain. The PHB content in the pta mutant harboring phbCAB was approximately twice those of the respective control strains. On LB supplemented with 20 g of glucose liter\(^{-1}\), the PHB content of JP231 harboring pKT230 reached 24.5% of dry cell weight, while that of W3110 harboring the same plasmid was only 14.7%. This result indicates that the perturbed acetyl CoA flux was indeed redirected to PHB synthesis.

Since the perturbed acetyl CoA flux in the pta mutant can be directed to the synthesis of PHB, it might be expected that the introduction of PHB synthesis pathway genes would affect the maximum growth rate of the pta mutant. Recombinant strain JP231 with phbCAB was found to grow faster than a recombinant strain harboring pKT230, a control vector, on either TB or TB supplemented with 2 g of glucose liter\(^{-1}\) (Table 3). In comparison, the recombinant strain W3110 harboring either phbCAB or its vector grew slightly slower than the vector-free control strain. These results showed that the defective growth of the pta mutant is recovered, at least in part, by redirecting the acetyl CoA flux to PHB synthesis. In order to check whether this improved growth is strain specific, a comparison was made between the RR1 strain and its pta mutant (JP202), both harboring either plasmid pKT230 or pSK2665, a high-copy-number plasmids containing phbCAB (38). Recovery from the growth defect was more pronounced in JP202 (RR1 pta mutant) with pSK2665, showing a 1.2-fold-higher growth rate than the control strain on LB, while the growth rate of JP202 harboring pTKPHB remained unchanged. The growth rates of RR1 strains harboring either plasmid pKTPHB or pSK2665 decreased to 90% of that of the control strain (data not shown).

Starvation survival defect of the pta mutant is also recovered by the introduction of phbCAB. One of the most prominent phenotypes of the pta mutant is its poor starvation survival, which was proposed to be a result of its inability to provide acetyl phosphate (30). Encouraged by the finding that the introduction of phbCAB genes repressed the defective growth of the pta mutant, we examined the effect of the introduction of phbCAB or its vector on the survival of the pta mutant in LB medium supplemented with 0.4 M NaCl. The survival rate of the pta mutant harboring the vector was significantly lower than that of the control strain, while the survival rate of the pta mutant harboring phbCAB was comparable to that of the control strain (data not shown).

### Table 2. Specific growth rates of E. coli W3110 and its pta mutant (JP231) on various carbon sources

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Specific growth rate (h(^{-1})) of:</th>
<th>W3110</th>
<th>JP231</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB</td>
<td>1.30 ± 0.05</td>
<td>0.59 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.59 ± 0.05</td>
<td>0.49 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>0.44 ± 0.02</td>
<td>0.33 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.38 ± 0.03</td>
<td>0.17 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.41 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>0.31 ± 0.03</td>
<td>0.30 ± 0.04</td>
<td></td>
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</tbody>
</table>

* The culture medium contained M9 salts with 2 g of each carbon source liter\(^{-1}\). Results are the means of three independent experiments.
of phbCAB genes on the starvation survival of the pta mutant. W3110, JGP231, and their recombinant strains harboring pKTPHB were cultivated in M9 minimal medium with 0.2 g of glucose liter⁻¹. After the depletion of glucose, incubation was continued for 9 days and viable cells were counted as colonies plated on LB plates after appropriate dilution (30). As shown in Fig. 5, the viability of JP231 harboring phbCAB genes was indistinguishable from that of the wild-type strain while the pta mutant showed poor survival. This result indicates that the defective starvation survival of the pta mutant could also be recovered by redirecting acetyl CoA flux to PHB synthesis, demonstrating that managing the acetyl CoA flux rather than providing acetyl phosphate is important for the survival of E. coli under starvation conditions.

**DISCUSSION**

In this study, we showed that the pta mutation resulted in a growth defect and the excretion of unusual by-products. In order to illustrate further the metabolic basis of phenotypic changes in the pta mutant, the stoichiometric method of flux analysis by Holms (19) was adopted with a slight modification in that the TCA cycle does not function in the cyclic mode. Components of the TCA cycle function almost exclusively to provide three precursor metabolites, namely, oxaloacetate (OAA), α-ketoglutarate, and succinyl CoA (29). The TCA cycle does not function as an energy-generating cycle. Moreover, in a glucose medium, the expressions of the enzymes in the TCA cycle are repressed, and, especially, α-ketoglutarate dehydrogenase activity was absent (2, 31, 41). In Fig. 6a, which shows a flux distribution of the precursor metabolites required for the synthesis of 1 g of biomass, the flux to PEP is insufficient and the insufficiency is resolved hypothetically by postulating a reverse flux through PEP synthetase, indicating the oversupply of pyruvate. A more likely way of supplying sufficient PEP flux is the uptake of additional glucose and its conversion to acetate, which is illustrated in Fig. 6b. In this case, it was assumed that 15% of the input glucose was excreted as acetate, reflecting the typical acetate yield of E. coli cultured on glucose. Now no hypothetical reverse flux from pyruvate to PEP is required. However, glucose transportation via PTS and the action of pyruvate kinase generate pyruvate (12.864 mmol) in excess of the required amount for precursor metabolites downstream of pyruvate, namely, acetyl CoA, α-ketoglutarate, and pyruvate (7.659 mmol). Oversupplied pyruvate and consequently acetyl CoA cannot be oxidized completely to CO₂ by the TCA cycle due to the repression of the TCA cycle enzymes, particularly of α-ketoglutarate dehydrogenase (2, 31, 41). Therefore, the pyruvate flux should be balanced by opting for acetate excretion (12). In the pta mutant, acetyl CoA flux is perturbed in

**TABLE 3. Partial recovery of growth defect of E. coli W3110 pta mutant (JP231) by introduction of phbCAB genes from A. eutrophusa**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific growth rate (h⁻¹) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB</td>
</tr>
<tr>
<td>W3110(pKT230)</td>
<td>1.29 ± 0.01</td>
</tr>
<tr>
<td>W3110(pKTPHB)</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>JP231(pKT230)</td>
<td>0.62 ± 0.01</td>
</tr>
<tr>
<td>JP231(pKTPHB)</td>
<td>0.67 ± 0.03</td>
</tr>
</tbody>
</table>

a The recombinant strains were grown on TB or TB supplemented with 2 g of glucose liter⁻¹. Results are the means of three independent experiments.

**FIG. 5. Starvation survival of W3110, JP231, and their recombinant strains harboring phbCAB genes from A. eutrophus.** Strains were grown in shaking flasks containing M9 minimal medium supplemented with 0.2 g of glucose liter⁻¹. After growth was arrested, incubation was continued for 9 days under the same condition. Viable cells were counted as colonies in LB plates after appropriate dilutions. One hundred percent viability corresponds to the number of viable cells counted 1 h after entering starvation. Symbols: ○, W3110; ●, W3110(pKTPHB); □, JP231; ■, JP231(pKTPHB).
The flux values in parentheses in Fig. 6b illustrate the theoretical flux distribution in the pta mutant, showing that the reduced acetate flux was redirected to the excretion of pyruvate, D-lactate, and L-glutamate. Excretion of pyruvate is a well-known characteristic of a mutant with a defective Pta-AckA pathway (12, 21). The accumulated acetyl CoA inhibits the activity of the pyruvate dehydrogenase complex, which causes the accumulation of pyruvate. Excretion of D-lactate may be the result of an elevated expression of D-LDH as well as pyruvate accumulation. In this regard, it is notable that the expression of ldhA, encoding fermentative D-LDH, increased and became independent of the medium pH by the mutations in the pta gene (7). Another possible response of a pta mutant to solve the problem involves the redirection of the acetyl CoA flux into the TCA cycle. Increased acetyl CoA flux into the TCA cycle requires an equivalent increase of PEP flux to OAA. The increased carbon flux must be excreted as by-products such as intermediates of the TCA cycle or amino acids if the TCA cycle does not function in a cyclic mode. As expected, the pta mutant excreted glutamate in an aerobic cultivation on glucose minimal medium or TB. Atkinson and Ninfa’s recent finding that higher activity of glutamine synthetase increased the flux from α-ketoglutarate to glutamate and resulted in a decreased level of acetyl phosphate (4) is consistent with glutamate accumulation in the pta mutant. The excretion of glutamate confirms that α-ketoglutarate dehydrogenase is repressed during aerobic growth on glucose or amino acids (2, 31, 41). The flux to pyruvate, D-lactate, and glutamate in the pta mutant was equivalent to the reduction in acetate flux compared to that for the wild-type strain (Fig. 1b and 6b).

Why does the pta mutant grow slower than the wild-type strain if flux balance is accomplished by excreting pyruvate, D-lactate, and glutamate instead of acetate? Since acetate metabolism has been proposed to function as a secondary energy-yielding pathway, when the TCA cycle or respiration capacity or both are saturated (3), reduced energy generation due to the pta mutation may be responsible for the growth defect. However, the amount of ATP generated by the pta mutant was calculated as 73.425 mM g (dry weight)\(^{-1}\), which reached 90.5% of the total energy generated by the wild type (81.125 mM g [dry weight]\(^{-1}\)). In this calculation, it was assumed that all NADH generated was converted to ATP and that the P/O ratio was 1.5. Moreover, at higher growth rates, sufficient energy (ATP) is available from the Embden-Meyerhof-Parnas (EMP) pathway to minimize the energy-producing role of the TCA cycle (22). Therefore, a reduction in ATP generation cannot explain the large growth defect of the pta mutant, although the possibility of its partial contribution cannot be excluded. It is more plausible that the retarded growth on several carbon sources is due to the perturbation in the fluxes of acetyl CoA and pyruvate, which in turn would affect the substrate transport rate. In the pta mutant grown on glucose, the accumulation of pyruvate will lower the PEP/pyruvate ratio, which results in a lowered glucose uptake rate (25, 32). Moreover, accumulated acetyl CoA activates PEP carboxylase (Ppc), a PEP-consuming enzyme, which will decrease available PEP for glucose transport (20). Glutamate excretion in the pta mutant reflects the increased flux through Ppc. The slow uptake of substrate was also observed in the cultivation of the pta mutant on TB, which is demonstrated by the lags between the assimilation of amino acids and the disappearance of glycine accumulation. The retardation of the amino acid assimilation of the pta mutant is comparable to that of nruo mutants; a nruo mutant, defective in membrane-bound NADH dehydrogenase I, consumed L-glutamate slowly and glycine, L-threonine, and L-alanine poorly at the entry into stationary phase (34).
slow or poor consumption of the amino acids was proposed to be a result of a higher NADH/NAD\(^+\) ratio, inhibiting both TCA cycle enzymes and enzymes functioning in the assimilation of amino acids (34). The \(pta\) mutation would decrease substrate uptake, and this in turn would lower the rate of growth.

If the growth defect of the \(pta\) mutant is caused by an unbalanced flux of acetyl CoA, the introduction of any pathway that can convert acetyl CoA to cell components or other metabolic end products should restore the growth of the \(pta\) mutant. As expected, when \(phbCAB\), the PHB synthesis genes from \(A.\ eutrophus\), were introduced, the growth defect of the \(pta\) mutant was restored (Table 3 and Fig. 5). The PHB content in the \(pta\) mutant was two times higher than that of the wild type, which demonstrated a redirection of the acetyl CoA flux normally destined for acetic acid synthesis. The difference between the PHB synthetic fluxes from acetyl CoA of the \(pta\) mutant (6.23 mmol) and the wild-type strain (3.31 mmol) was 2.92 mmol or 86% of the reduction of acetic acid flux (3.38 mmol), which shows that the increased flux directed to PHB synthesis actually contributes to the management of acetyl CoA flux.

Diaz-Ricci et al. reported similarly that the expression of pyruvate decarboxylase and alcohol dehydrogenase genes from \(Zymomonas\\, mobilis\) overcame the metabolic stress caused by the plasmid, enhancing growth and glucose uptake rates of a \(pta\) mutant to the observed values for the plasmid-free \(pta\) mutant (12). Particularly notable is that the starvation survival of the \(pta\) mutant was recovered by \(phbCAB\) introduction, which suggested that the poor survival of the \(pta\) mutant was due to problems of acetyl CoA flux rather than its inability to synthesize acetyl phosphate.

Our conclusion is that aerobic acetate production in \(E.\ coli\) is due to the oversupply of pyruvate from PTS, which strongly suggests that the production of acetate is a function of the balance of metabolic flux and not of the central metabolic rate or the growth rate itself. If the production of acetate is a matter of balance, why is acetate accumulation eliminated at lower growth rates? Two explanations are possible. First, the elimination of acetate excretion at lower growth rates may be due to the change in the glucose uptake system. Transport of substrate through the non-PTS system does not require a massive conversion of PEP to pyruvate, which may eliminate the pyruvate oversupply and in turn the excretion of acetate. In this context, it is worth noting that the non-PTS glucose uptake systems such as the maltose or galactose transport systems were found to be derepressed at lower growth rates in continuous culture (10, 11). Recently, Chen et al. showed that when \(E.\ coli\) exclusively transports glucose by the galactose transport system, a non-PTS Na symport system, the aerobic production of acetate was totally eliminated (9), which supports our contention that aerobic acetate production depends on the nature of the glucose uptake system. Second, the elimination of acetate excretion at lower growth rates may be the effect of derepression of TCA cycle enzymes, which permits a further metabolism of acetyl CoA. As TCA cycle enzymes are derepressed at lower growth rates in continuous culture, oversupplied pyruvate and acetyl CoA may be metabolized to CO\(_2\) via the TCA cycle and acetate excretion may be eliminated. Further studies to examine the effects of changes of the glucose transport system or derepression of TCA cycle enzymes on acetate metabolism are in progress.

Another intriguing question arises here: why is the PTS system chosen as the principal substrate uptake system if acetate excretion is due to PTS? Does PTS offer a competitive advantage in terms of outgrowth competition? Because the Mgl system coupled with LamB glycoporine appears to support slower growth (15), it is tempting to suggest that the PTS system is used to achieve maximum growth rate at the expense of the growth yield due to acetate excretion. It is worth noting that a mutant with a higher glucose transport capacity evolved as one of the major populations during a long-term culture of \(E.\ coli\) in glucose-limited continuous culture (37). Furthermore, excreted acetate may be reused during the stationary growth phase when the principal substrates are exhausted (24), allowing cells to adapt to and survive starvation conditions.

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