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Common Enzymes of Branched-Chain Amino Acid Catabolism in *Pseudomonas putida*

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Two types of *Pseudomonas putida* PpG2 mutants which were unable to degrade branched-chain amino acids were isolated after mutagenesis and selection for ability to grow on succinate, but not valine, as a sole source of carbon. These isolates were characterized by growth on the three branched-chain amino acids (valine, isoleucine, and leucine), on the corresponding branched-chain keto acids (2-ketoisovalerate, 2-keto-3-methylvalerate, and 2-ketoisocaproate), and on other selected intermediates as carbon sources, and by their enzymatic composition. One group of mutants lost 2-ketoisovalerate-inducible branched-chain keto acid dehydrogenase that was active on all three keto acids. There was also a concomitant loss of ability to grow on all three branched-chain amino acids as well as on all three corresponding keto acids, but there was retention of ability to use subsequent intermediates in the catabolism of branched-chain amino acids. Another type of mutant showed a marked reduction in branched-chain amino acid transaminase activity and grew poorly at the expense of all three amino acids, but it utilized subsequent intermediates as carbon sources. Both the transaminase and branched-chain keto acid dehydrogenase mutants retained the ability to degrade camphor. These findings are consistent with the view that branched-chain amino acid transaminase and branched-chain keto acid dehydrogenase are common enzymes in the catabolism of valine, isoleucine, and leucine.

Because purified D-amino acid dehydrogenase (8) and branched-chain amino acid transaminase (11) are capable of deaminating the D- and L-isomers, respectively, of all three branched-chain amino acids, it was thought possible that the degradation of these amino acids involves common enzymes. To understand the regulation of branched-chain amino acid catabolism, one must know whether valine, isoleucine, and leucine share a common pathway or whether each amino acid has a discrete pathway. The proposed pathways for the oxidation of the branched-chain amino acids (9; L. K. Massey and J. R. Sokatch, and R. S. Conrad and J. R. Sokatch, unpublished data) and the abbreviations for the relevant enzymes are presented in Fig. 1.

In this investigation, evidence for the existence of a common pathway was obtained by the isolation and characterization of mutants that are unable to use the branched-chain amino acids as carbon and energy sources. These mutants made it possible to determine whether a single genetic block involved all three branched-chain amino acids, and they provided a genetic approach for studying the regulation of valine catabolism in *Pseudomonas putida* (9).

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MATERIALS AND METHODS

Bacterial strains. *P. putida* strain PpG2 (CIS; ATCC 23287), obtained from I. C. Gunsalus, and mutants derived from it (Table 1) were used throughout these studies. A mutant phenotype is indicated by an isolation number. The nomenclature used follows the recommendations of Demerec et al. (2). Phenotype abbreviations (Table 1), rather than genotype symbols, have been used to identify mutants because the genes affected by the mutations have not been determined. Branched-chain amino acid transami-

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nase (EC 2.6.1.6) catalyzes the deamination of the L-series of branched-chain amino acids before their oxidation. Therefore, the phenotype of a mutant with a defective transaminase is designated BauA. Branched-chain keto acid dehydrogenase is the first enzyme unique to branched-chain amino acid catabolism in *P. putida*. The phenotype of a mutant without branched-chain keto acid dehydrogenase is designated BauB. For the sake of clarity and completeness, phenotype abbreviations have also been assigned (Fig. 1) for the traits expected of mutants which have as yet not been isolated.

Media. Cultivation was in the basal medium (medium G) described by Jacobson et al. (3). The carbon sources were sterilized separately by filtration through a membrane filter (0.22 μm pore size) and added aseptically.

The concentration of each of the carbon sources present and the corresponding optimal pH for its utilization are shown in Table 2. When (+)-camphor was used as a carbon source, a sample (0.1 ml) of a 2-M solution in methylene chloride was spread on the lid of a petri plate and allowed to dry before incubation.

Growth conditions. The following procedure was used in culturing cells for nutritional studies. An inoculum from a 2% nutrient agar stock slant was grown in 2 ml of medium E (18) containing 0.2% Casamino Acids and 0.5% glucose for 18 to 20 h. A transfer was made to 2 ml of medium G containing 0.5% disodium succinate or 0.5% sodium glutamate. The culture was incubated for approximately 14 h, centrifuged, and suspended in 2 ml of sterile dilution buffer (medium G without added carbon source). To determine the relative abilities of the mutants and wild type (PpG2) to grow on a carbon source, portions (0.1 ml) of appropriate dilutions were spread (50 to 150 colonies per plate) onto solid medium G supplemented with the carbon source to be tested. The plates were incubated for 72 h. The size of isolated mutant colonies on a test carbon source was compared with that of wild-type colonies grown on the same compound. Growth was scored as minus (significantly less than that of wild type) or plus (equal to that of wild type). Growth of cultures in liquid medium on a rotary shaker was measured turbidimetrically with a Klett-Summerson colorimeter fitted with a no. 66 filter. The number of viable cells was estimated from a previously prepared standard curve relating the number of viable cells to absorbancy. All cultures were grown at 30 C.

Isolation of mutants. Washed, exponentially growing cells (10^8 to 2×10^8 per ml) from medium G with succinate (MG + succ) were treated with 100 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine per ml for 20 min at 37 C by the method of Adelberg et al. (1), except that medium G without carbon source was substituted for the minimal medium as a component of the tris(hydroxymethyl)aminomethane-maleate buffer. Alternatively, mutagenesis was effected in dilution buffer with 0.35 M ethyl methane sulfonate (6). A sample of the mutagenized culture was inoculated into 5 ml of MG + succ and incubated with aeration for 24 h. The cells were centrifuged, washed once with 5 ml of dilution buffer, resuspended to give a Klett-Summerson reading of 5 to 10 in 20 ml of medium G containing 0.5% L-valine and 0.005% L-isoleucine (MG + V + ile) in a 250-ml Erlenmeyer flask fitted with a Klett tube, and incubated with aeration. Penicillin (50,000 U/ml) was added after growth had proceeded to a Klett reading of 20 to 25, and the culture was reincubated for 8 h. The cells were harvested by centrifugation, washed once with 20 ml of dilution buffer, and resuspended in 20 ml of this buffer.

TABLE 1. *Strains of Pseudomonas putida PpG2*

Strain	Phenotype	Derivation[a]
PpG2.	Wild type	I. C. Gunsalus
PpM2106	BauA	EMS of PpG2
PpM2109	BauB	NTG of PpG2
PpM2111	BauB	NTG of PpG2
PpM2120	BauB	NTG of PpG2
PpM2124	BauB	NTG of PpG2

[a] EMS, treatment with ethyl methane sulfonate; NTG, treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Portions (0.1 ml) of suitable dilutions of this suspension were spread onto triplicate plates of MG + succ agar. After 48 h of incubation, colonies were transferred by replica plating first onto MG + V + ile agar, and then onto MG + succ agar and incubated for 48 h. Mutants lacking an obligate enzyme for valine degradation were unable to grow on valine but were able to utilize succinate as a sole source of carbon.

Enzyme assays. Extracts were prepared as described by Marshall and Sokatch (9) and used for the determination of enzyme activities.

For branched-chain amino acid transaminase (EC 2.6.1.6), the assay of Taylor and Jenkins (16) was used. Activity is expressed as nanomoles of 2-ketoisovalerate formed (from valine) per minute per milligram of protein. The transaminase assay is able to detect a lower limit of 5 to 10 nmol of the keto acid produced per min.

Branched-chain keto acid dehydrogenase was measured by the procedure of Marshall and Sokatch (9). The activity of 3-hydroxyisobutyrate dehydrogenase (EC 1.1.1.31) was determined according to the procedure of Robinson and Coon (13), and that of methylmalonate semialdehyde dehydrogenase by the method of Sokatch, Sanders, and Marshall (15). Activities for the three dehydrogenases are expressed as nanomoles of nicotinamide adenine dinucleotide, reduced form (NADH), formed from NAD per minute per milligram of protein. These kinetic assays are able to detect as little as 30 nmol of NADH produced per min.

Protein content of the extracts was determined by the method of Warburg and Christian (19).

RESULTS

Inhibition by branched-chain amino acids of P. putida PpG2 growth. Isoleucine overcomes valine inhibition of growth of *Escherichia*

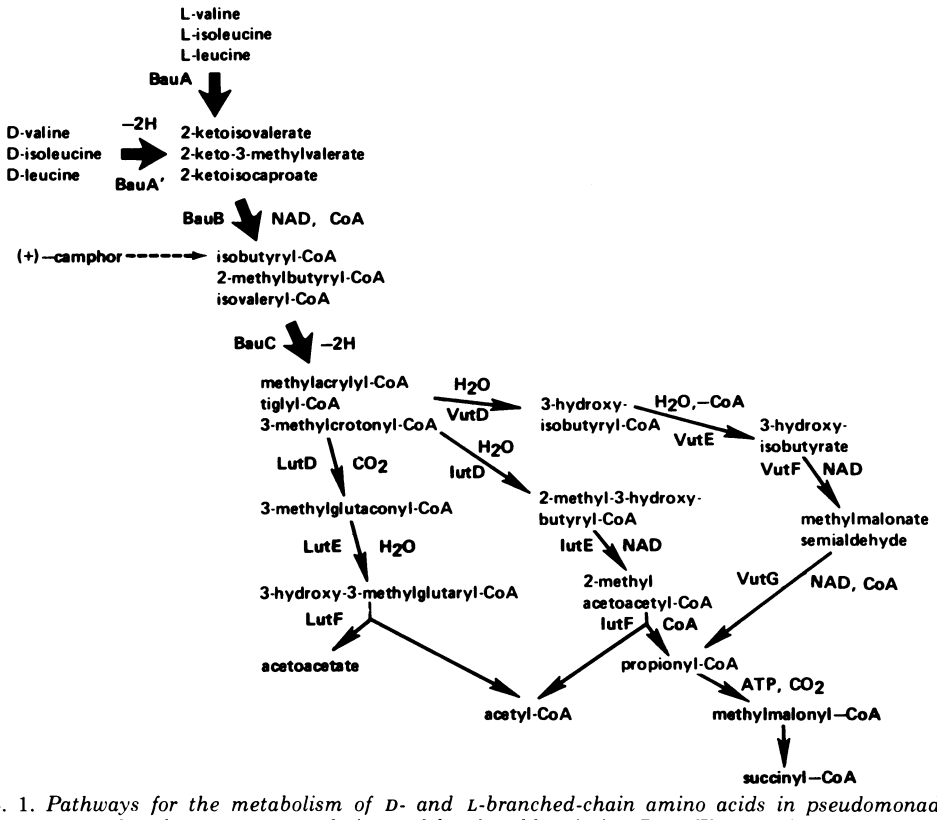

FIG. 1. *Pathways for the metabolism of* D- *and* L-*branched-chain amino acids in pseudomonads. The enzymes proposed to be common are designated by the abbreviation Bau. The reactions they catalyze are represented by wide arrows. Enzymes thought to be unique to leucine, isoleucine, or valine utilization are designated by the symbols Lut, iut, or Vut, respectively. BauA' and BauC are* D*-amino acid dehydrogenase and isobutyryl-coenzyme A dehydrogenase, respectively. The enzymatic reactions and enzymes whose activities were measured in this study are: BauA, branched-chain amino acid transaminase; BauB, branched-chain keto acid dehydrogenase; VutF, 3-hydroxyisobutyrate dehydrogenase; and VutG, methylmalonate semialdehyde dehydrogenase.*

TABLE 2. *Conditions for growth of Pseudomonas putida PpG2 on selected carbon sources*

Carbon source	Concn (mg/ml)	Additional supplement (50 μg/ml)	pH
L-Valine	5.0	L-Isoleucine	7.0
L-Isoleucine	5.0	L-Leucine + L-valine	7.0
L-Leucine	5.0	L-Valine	7.0
2-Ketoisovalerate	4.0	None	8.0
2-Keto-3-methylvalerate	4.0	None	8.0
2-Ketoisocaproate	4.0	None	8.0
Isobutyrate	0.5	L-valine + L-isoleucine + L-leucine	7.0
2-Methylbutyrate	0.5	L-valine + L-isoleucine + L-leucine	7.0
Isovalerate	0.5	L-valine + L-isoleucine + L-leucine	7.0
3-Hydroxyisobutyrate	4.0	None	7.0
Propionate	2.5	None	7.0
Methylmalonate	7.5	None	6.5
Succinate	5.0	None	7.0
Glutamate	5.0	None	7.0

coli K-12 (17), *Salmonella typhimurium* (12), *Acetobacter suboxydans* (5), and *P. putida* (L. A. Jacobson, Ph.D. thesis, Univ. of Illinois, Urbana, 1967), and the inhibition by leucine of relaxed and stringent strains of *E. coli* K-12 (14). We also observed that valine inhibits the growth of *P. putida* and that isoleucine overcomes this toxicity. In addition, the effect of leucine and isoleucine on growth has been examined.

Colony size was markedly reduced when each of the branched-chain amino acids at a concentration of 5.0 mg/ml was used individually as a sole source of carbon (Table 3). A small amount of L-isoleucine (final concentration, 50 μg/ml) relieved the inhibition by valine. Therefore, media containing valine as a sole carbon source were normally supplemented with 50 μg of L-isoleucine per ml. Whereas L-valine alone was effective in stimulating growth on leucine, both L-valine and L-leucine were required to achieve the same degree of reversal of inhibition by isoleucine. Therefore, valine was routinely added to leucine-containing media, and a mixture of valine and leucine was supplied in isoleucine-containing media. The concentration (50 μg/ml) of the supplement used to reverse inhibition was not by itself sufficient to support significant growth.

Elucidation of the defect in the valine catabolic pathway. Mutants able to utilize succinate, but not valine, as a sole source of carbon were presumed to be defective in one of the valine-degrading enzymes. The genetic block was characterized by growth of the mutants at the expense of the pathway intermediates, and by enzyme assay of extracts prepared from cells grown under conditions in which the corresponding enzymes in the wild type were present. Two types of mutants were found (Table 4). One type, strain PpM2106, could not degrade L-valine, and showed slightly impaired growth on 2-ketoisovalerate and normal growth on isobutyrate, 3-hydroxyisobutyrate, propionate, methylmalonate, and succinate as carbon sources. Transaminase activity in the mutant was 30 to 40% of that in PpG2, whereas the levels of the other enzymes appeared to be normal (Table 5). The apparent reduction in dehydrogenase activity to 10% of the fully

TABLE 3. *Inhibition by branched-chain amino acids of growth of Pseudomonas putida PpG2*

Carbon source (5.0 mg/ml)	Supplement (50 μg/ml)	Relative colony size[a]
L-Valine		+
L-Valine	L-Leucine	++
L-Valine	L-Isoleucine	++++
L-Valine	L-Isoleucine + L-leucine	++++
L-Isoleucine		++
L-Isoleucine	L-Valine	+++
L-Isoleucine	L-Leucine	+++
L-Isoleucine	L-Valine + L-leucine	++++
L-Leucine		++
L-Leucine	L-Isoleucine	++
L-Leucine	L-Valine	++++
L-Leucine	L-Isoleucine + L-valine	++++

[a] Colony size (48 h) compared to that on succinate, which was assigned a value of 10.

TABLE 4. *Ability of Bau mutants of Pseudomonas putida PpG2 to grow on carbon sources related to the metabolism of branched-chain amino acids*

Carbon source	Strain					
	PpG2	PpM2106	PpM2109	PpM2111	PpM2120	PpM2124
Valine	+	−	−	−	−	−
Isoleucine	+	−	−	−	−	−
Leucine	+	−	−	−	−	−
2-Ketoisovalerate	+	+[a]	−	−	−	−
2-Keto-3-methylvalerate	+	+	−	−	−	−
2-Ketoisocaproate	+	+	−	−	−	−
Isobutyrate	+	+	+	+	+	+
2-Methylbutyrate	+	+	+	+	+	+
Isovalerate	+	+	+	+	+	+
3-Hydroxyisobutyrate	+	+	+	+	+	+
(+)-Camphor	+	+	+	+	+	+
Propionate	+	+	+	+	+	+
Methylmalonate	+	+	+	+	+	+
Succinate	+	+	+	+	+	+

[a] Impaired growth.

TABLE 5. *Activities of valine catabolic enzymes in Bau mutants of Pseudomonas putida PpG2*

Enzyme	Relative specific activity[a] after growth on 0.3% L-glutamate and 0.1% L-valine					
	PpG2	PpM2106	PpM2109	PpM2111	PpM2120	PpM2124
Branched-chain amino acid transaminase	1.0	0.3	0.7	1.4	1.1	0.8
Branched-chain keto acid dehydrogenase	1.0	0.1	0	0	0	0
3-Hydroxyisobutyrate dehydrogenase	1.0	0.7	0.7	0.6	0.5	0.7
Methylmalonate semialdehyde dehydrogenase	1.0	1.6	0.9	0.6	1.0	1.2

[a] Specific activity of the mutant enzyme compared to the activity of the enzyme from strain PpG2, which was assigned a value of 1.0.

induced wild-type level was presumably due to inefficient formation from valine of the actual inducer of the enzyme 2-ketoisovalerate (9). Since the dehydrogenase could be readily induced to normal levels by growth in the presence of 2-ketoisovalerate, 2-keto-3-methylvalerate, or 2-ketoisocaproate (9), this enzyme was actually unaffected by the mutation in PpM2106. It was concluded that PpM2106 is unable to efficiently convert valine to 2-ketoisovalerate because of a partial block in branched-chain amino acid transaminase (BauA).

The second type of mutant, of which PpM2124 is representative, grew on isobutyrate and subsequent intermediates, but utilized neither valine nor 2-ketoisovalerate as carbon sources (Table 4). Extracts from cells of strain PpM2124 grown with added valine were lacking detectable branched-chain keto acid dehydrogenase activity but contained normal levels of the other enzymes that were assayed (Table 5). Moreover, it was not possible to induce the synthesis of branched-chain keto acid dehydrogenase even by growth in the presence of the natural inducer 2-ketoisovalerate (Table 6). Therefore, this mutant differed from the BauA mutant in that it was unable to grow on the keto acids as a sole source of carbon (Table 4), and the keto acid dehydrogenase was not inducible even by growth in the presence of 2-ketoisovalerate (Table 6). It was concluded that PpM2124 is unable to convert 2-ketoisovalerate to isobutyryl coenzyme A because of a defective branched-chain keto acid dehydrogenase (BauB). Strains 2109, 2111, and 2120 are also considered to be branched-chain keto acid dehydrogenase mutants.

TABLE 6. *Noninducibility by valine and 2-ketoisovalerate of branched-chain keto acid dehydrogenase in BauB mutants of Pseudomonas putida PpG2*

Strain	Inducer	Specific activity[a]
PpG2	None[b]	0
	D, L-Valine[c]	94
	2-Ketoisovalerate[b]	83
PpM2111	None	0
	D, L-Valine	0
	2-Ketoisovalerate	0
PpM2124	None	0
	D, L-Valine	0
	2-Ketoisovalerate	0

[a] Values shown indicate nanomoles of product formed per minute per milligram of protein.

[b] Cells were grown in the presence of 0.3% L-glutamate.

[c] Present at a concentration of 0.1%. Cells were grown in the presence of 0.01% D, L-isoleucine and 0.3% L-glutamate.

Common enzymes appearing early in branched-chain amino acid catabolism. A defect in an enzyme shared by the pathways for catabolism of the branched-chain amino acids should lead to the absence or reduction of an analogous enzyme activity in each of the three pathways, and to a simultaneous inability to grow on valine, isoleucine, and leucine, as well as on the respective intermediates preceding the block.

To determine whether the three branched-chain amino acids share early degradative enzymes, the Bau mutants were further tested for concomitant inability to grow at the expense of all three branched-chain amino acids, their respective keto and branched-chain fatty acids, 3-hydroxyisobutyrate, and several subsequent metabolites.

The transaminase mutant PpM2106 showed little ability to utilize either isoleucine, leucine, or valine as carbon sources, but it retained ability to degrade subsequent intermediates in all three branched-chain amino acid catabolic pathways (Table 4). The fact that PpM2106 was pleiotropic, together with the finding that branched-chain amino acid transaminase was the only enzyme measured whose activity was markedly reduced (Table 5), is consistent with the view that this transaminase is an enzyme functionally shared by all three degradative pathways. This concept is supported by the observation that purified branched-chain amino acid transaminase of *P. aeruginosa* is capable of catalyzing the deamination of all three branched-chain amino acids (11).

The BauB mutants, PpM2109 through 2124, showed a simultaneous inability to grow at the expense of all three branched-chain amino acids as well as all three corresponding keto acids (Table 4); additionally, extracts did not oxidize 2-ketoisovalerate, 2-keto-3-methylvalerate, or 2-ketoisocaproate (Table 7). These mutants utilized subsequent intermediates of the valine, isoleucine, and leucine pathways as carbon sources (Table 4), contained all of the other degradative enzymes measured (Table 5), and oxidized other keto acids such as 2-ketoglutarate and pyruvate (Table 7).

That branched-chain keto acid dehydrogenase is an enzyme shared by the valine, isoleucine, and leucine degradative pathways appears to be a logical extension of the findings.

Since isobutyrate is a common product of both valine and camphor catabolism, it was of

interest to test the ability of the mutants to grow at the expense of (+)-camphor. The genes specifying enzymes which function early in the metabolism of camphor are located solely on a transmissible plasmid (J. G. Rheinwald, M. S. thesis, Univ. of Illinois, Urbana, 1970).

Therefore, retention by both the transaminase and branched-chain keto acid dehydrogenase mutants of the ability to utilize camphor as a carbon source (Table 4) strongly suggests that the plasmid is still present.

Retention by strains of *P. putida*, cured of the camphor plasmid, of the ability to grow on the branched-chain amino and keto acids (H. Dunn and I. C. Gunsalus, and R. R. Martin, J. R. Sokatch, and L. Unger, unpublished data) and the occurrence of transaminase and dehydrogenase mutants which still carry the plasmid further suggests that the genes specifying these enzymes are located on the chromosome and not on the extrachromosal element.

DISCUSSION

Among the mutants deficient in the ability to utilize L-valine as a sole source of carbon, a strain (PpM2106) was found which had a specific marked reduction (30 to 40%) in branched-chain amino acid transaminase activity (Table 5). Mutant PpM2106 was unable to grow at the expense of valine but it did grow on subsequent intermediates in the valine catabolic pathway (Table 4). Branched-chain amino acid transaminase appears to participate both in the last step in the biosynthesis of valine from 2-ketoisovalerate and in the first step in the catabolism of valine to 2-ketoisovalerate. Purified branched-chain amino acid transaminase from *P. aeruginosa* catalyzes the reversible transfer of amino group from each of the three branched-chain amino acids to 2-ketoglutarate (11). If a single transaminase functions in both a biosynthetic and catabolic capacity, then a mutation in the corresponding gene might be expected to create a requirement for branched-chain amino acids for growth. However, mutant PpM2106 grew normally on succinate as a sole source of carbon and showed no apparent growth response to added valine, isoleucine, or leucine. The finding of a mutant which is unable to oxidize the three branched-chain amino acids, but does not require these amino acids, suggests the presence of residual nonspecific transaminases or the possibility of two functionally distinct branched-chain amino acid transaminases: a biosynthetic enzyme and a catabolic enzyme. If the latter is the case, the catabolic transasminase is defective in the BauA mutant. In this connection, it is noteworthy that it has not been possible to isolate transaminase B (*ilvE*) mutants of *P. aeruginosa* (7).

TABLE 7. *Simultaneous loss by BauB mutant PpM2124 of ability to oxidize all three branched-chain keto acids*

Substrate	Specific activity of branched-chain keto acid dehydrogenase[a]	
	PpG2	PpM2124
2-Ketoisovalerate	79	4
2-Keto-3-methylvalerate	59	0
2-Ketoisocaproate	93	0
2-Ketoglutarate	300	290
Pyruvate	49	37

[a] Values shown indicate nanomoles of product formed per minute per milligram of protein.

In the wild type, branched-chain keto acid dehydrogenase was induced by the keto acids formed endogenously as a result of the catabolic transamination of added branched-chain amino acids (Tables 5 and 6) (9). However, in BauA mutant PpM2106, the enzyme remained uninduced during growth in the presence of valine (Table 5). Therefore, although residual transaminase activity was present in extracts of the BauA mutant, the block in the in vivo catabolic deamination of L-valine must have been tight enough to preclude the formation of sufficient keto acid to induce appreciable dehydrogenase activity and allow significant growth at the expense of valine. Therefore, if indeed there are two transaminases in *P. putida*, the biosynthetic enzyme apparently does not assume efficiently the role of the catabolic transaminase in vivo when the latter becomes nonfunctional.

The same phenotype might be expected to result from a mutation specifying an alteration in the affinity of the transaminase for the amino acid. Some insight into this concept can be gained from studying the published values for the concentration of branched-chain amino acids in the amino acid pool of *P. aeruginosa* (4) and the Michaelis constants for the purified branched-chain amino acid transaminase of *P. aeruginosa* (11). Branched-chain amino acid transaminase of *P. aeruginosa* should be more effective in catalyzing deamination, because the Michaelis constant for the keto acids is approximately one order of magnitude lower than it is for the amino acids. When branched-chain amino acids are the energy source for growth, the amino acid pool would be high and deamination would be significant. If a mutation occurred which reduced the binding efficiency of the transaminase for the amino acid. biosynthesis of branched-chain amino acids could

remain unaffected, whereas catabolism would be impaired.

There is good reason to believe that the early enzymes of the pathway in *P. putida*, D-amino acid dehydrogenase, branched-chain amino acid transaminase, and branched-chain keto acid dehydrogenase, are common to the metabolism of all three branched-chain amino acids. Purified D-amino acid dehydrogenase (8) and branched-chain amino acid transaminase (11) are capable of deaminating the D- and L-isomers, respectively, of all three branched-chain amino acids. The mutation which affected the transaminase in PpM2106 (Table 5) was associated with a concomitant loss of ability to grow at the expense of all three branched-chain amino acids (Table 4). The synthesis of branched-chain keto acid dehydrogenase is induced by each of the three branched-chain keto acids in PpG2 (9). Extracts containing branched-chain keto acid dehydrogenase catalyzed the oxidative decarboxylation of 2-ketoisovalerate, 2-keto-3-methylvalerate, and 2-ketoisocaproate (Table 7). BauB mutants such as PpM2124 showed a complete and apparently specific loss of keto acid-inducible ability to oxidize all three keto acids (Table 7) and a concomitant inability to grow on the three branched-chain amino acids as well as on their corresponding keto acids as carbon sources (Table 4).

Although not yet analyzed enzymatically, it is of interest that another class of mutants showed a phenotype which was consistent with a block in isobutyryl-coenzyme A dehydrogenase and with the concept that this enzyme is also shared by a pathway common for all three branched-chain amino acids. These mutants utilized neither the three branched-chain amino acids, the branched-chain keto acids, nor isobutyrate, 2-methylbutyrate, and isovalerate as carbon sources, but grew normally at the expense of subsequent intermediates such as 3-hydroxyisobutyrate.

An interesting feature of branched-chain amino acid metabolism is that the repressible biosynthetic and inducible degradative pathways each involve enzymes which are common to all three amino acids. It is possible that this property may provide one basis for regulation of the intracellular steady-state level of endogenous branched-chain amino acids.

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