Cloning and Sequence Analysis of the LPD-glc Structural Gene of *Pseudomonas putida*

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*Pseudomonas putida* is able to produce three lipoamide dehydrogenases: (i) LPD-glc, which is the E3 component of the pyruvate and 2-ketoglutarate dehydrogenase complexes and the L-factor for the glycine oxidation system; (ii) LPD-val, which is the specific E3 component of the branched-chain keto acid dehydrogenase complex and is induced by growth on leucine, isoleucine, or valine; and (iii) LPD-3, which was discovered in a *lpdG* mutant and whose role is unknown. Southern hybridization with an oligonucleotide probe encoding the highly conserved redox-active site produced three bands corresponding to the genes encoding these three lipoamide dehydrogenases. The complete structural gene for LPD-glc, *lpdG*, was isolated, and its nucleotide sequence was determined. The latter consists of 476 codons plus a stop codon, TAA. The structural gene for LPD-glc is preceded by a partial open reading frame with strong similarity to the E2 component of 2-ketoglutarate dehydrogenase of *Escherichia coli*. This suggests that *lpdG* is part of the 2-ketoglutarate dehydrogenase operon. LPD-glc was expressed in *Pseudomonas putida* JS348 from pHPlP4 which contains a partial open reading frame corresponding to the E2 component, 94 bases of noncoding DNA, and the structural gene for *lpdG*. This result indicates that *lpdG* can be expressed separately from the other genes of the operon.

Lipoamide dehydrogenases (8, 14, 45) are redox-active disulfide flavoproteins and are glutathione reductases (18), mercuric reductases (4, 22), trypanothione reductase (29), asparaginase dehydrogenase (46), bis-γ-glutamylcysteine reductase (38), and pantetheine 4',4''-diphosphate reductase (39). Redox-active disulfide flavoproteins use NADH or NADPH to reduce a disulfide bond and are homologues containing 1 mol of flavin adenine dinucleotide per subunit.

The reaction catalyzed by lipoamide dehydrogenases is E2-Lip(SH)2 + NADH $\rightarrow$ E2-LipSH + NADH + H+, where E2-Lip(SH)2 is the E2 component of a keto acid dehydrogenase multi-enzyme complex which contains a covalently bound lipoic acid. The E3 component, lipoamide dehydrogenase, catalyzes the NADH-linked oxidation of the lipoyl prosthetic group, allowing E2 to recycle (44). In the oxidation of glycine by *Pseudomonas putida*, LPD-glc is required for oxidation of H2, a polypeptide which contains lipoic acid (30).

*P. putida* contains structural genes for three lipoamide dehydrogenases, LPD-glc, LPD-val, and LPD-3. LPD-glc is thought to be constitutively produced, is the L-factor of the glycine oxidation system (30), and is the E3 component of the pyruvate and 2-ketoglutarate dehydrogenase multi-enzyme complexes (32). LPD-val is the specific E3 component of branched-chain keto acid dehydrogenase and is induced when *P. putida* (31) and *Pseudomonas aeruginosa* (21) are grown in medium containing branched-chain amino acids (21, 31). LPD-3 is expressed in *lpdG* mutants of *P. putida* (14), which have pyruvate dehydrogenase activity but no 2-ketoglutarate dehydrogenase activity. So far, the function of LPD-3 has not been discovered. *Escherichia coli* was thought to have only one lipoamide dehydrogenase, but a second one has recently been purified from an *lpd* mutant (25).

In *E. coli*, the structural gene for lipoamide dehydrogenase, *lpd*, is linked to the pyruvate dehydrogenase operon (37). It is not known whether the structural gene for the second lipoamide dehydrogenase of *E. coli* (25) is linked to an operon. In contrast, the structural genes for the lipoamide dehydrogenases of *Azotobacter vinelandii* (42) and *Pseudomonas fluorescens* (2) are part of the 2-ketoglutarate dehydrogenase operon.

The main objective of the present research was to clone and determine the nucleotide sequence of *lpdG*, the structural gene encoding LPD-glc, and to compare the amino acid sequence of LPD-glc with those of other lipoamide dehydrogenases, particularly those of *P. putida*. The second objective was to determine the operon to which *lpdG* is attached and to study its expression.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. pHPl contains a 5-kb *SphI* fragment of *P. putida* DNA which includes the complete *lpdG* gene. pHPl contains a 2-kb *PstI* insert from pHPl with the *lpdG* gene in the orientation opposite to that of the lac promoter in pUC series plasmids. pHPl contains the *P. putida* DNA fragment from pHPl inserted into the *HindIII* and *SacI* sites of the broad-host-range vector pKT240 (1) which contains a promoterless streptomycin resistance gene. *P. putida* JS348 is Km3 Str1, and *P. putida* JS348(pKT240) is Km3 Str'. pHPl4 was introduced into *P. putida* PpG2 by triparental mating (13).

**Media and growth conditions.** *E. coli* and *P. putida* were grown in L broth (19) at 37 and 30°C, respectively, with aeration. Antibiotics were added to the medium, depending on which plasmids the cells contained, as follows: cells containing the pUC series plasmids, 200 μg of ampicillin; cells containing pRK2013, 90 μg of kanamycin per ml; and cells containing pKT240-based plasmids, 50 μg of carbenicillin per ml or 2 mg of streptomycin per ml. The basal medium was described earlier (16, 33), and basal medium

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TABLE 1. Strains and plasmids used in this study

<table>
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<th>Strain or plasmid</th>
<th>Genotype or description</th>
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</tr>
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<td>This study</td>
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<td>promoter</td>
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<td>pHp4</td>
<td>lpdG&lt;sup&gt;+&lt;/sup&gt; Str&lt;sup&gt;R&lt;/sup&gt; pHp3 insert in</td>
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<td></td>
<td>pKTK24</td>
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<sup>a</sup> Gene designation of lpdG refers to LPD-glc of P. putida PpG2.
<sup>b</sup> Bethesda Research Laboratories.

Free of ammonium ion was obtained by omitting ammonium sulfate from the basal G solution. Synthetic media contained basal G solution and salts S plus carbon sources at the following concentrations: 10 mM glucose, 10 mM DL-lysine, 40 mM serine, 30 mM threonine, and 60 mM glycine. N-free basal G solution and Salts S was used in medium containing glucose and glycine. Pseudomonas Isolation Agar was obtained from Difco Laboratories.

**Enzymes and chemicals.** Restriction enzymes, kinases, and phosphatases were purchased from Promega Corporation, Madison, Wis. Radioactive materials used in these studies were purchased from New England Nuclear, Dupont Corp., Boston, Mass. [α<sup>32</sup>P]dCTP (3,000 Ci/mmol) was used for nick translation and nucleotide sequencing, [γ<sup>32</sup>P]ATP (150 μCi; specific activity, 3,000 Ci/mmol), and the mixture was incubated at 37°C for 30 min. The labeled nucleotide was separated from [γ<sup>32</sup>P]ATP, using a spin Sephadex G25/50 column.

**Library construction.** A limited restriction enzyme digest of P. putida chromosomal DNA by SpH<sub>1</sub> produced a majority of fragments in the 4- to 9-kb range. The DNA was mixed with a complete SpH<sub>1</sub> digest of pUC19 which had been treated with alkaline phosphatase with a chromosome-to-vector ratio of 3:1 and ligated with T4 ligase. The amount of total DNA in the ligation and transformations ranged from 0.02 to 0.5 μg. Ligation was done in a total volume of 69 to 79 μl containing 2 U of T4 ligase, and the mixture was left overnight at 14°C. E. coli TB1 cells were made competent by the CaCl<sub>2</sub> method (20). Selection for recombinants was achieved by growth on ampicillin.

** Colony hybridizations.** A total of 3,000 white colonies of E. coli TB1 cells containing the P. putida PpG2 SpH<sub>1</sub>-digested DNA fragments were plated per screening. The colonies were transferred and lysed by using GeneScreen Plus (New England Nuclear) according to the manufacturer’s instructions in a solution of 0.5 N NaOH and neutralized in 1.0 M Tris-HCl, pH 7.5. The nylon membranes were prehybridized with 5× Denhardt solution, 0.7 μg of salmon sperm DNA, 0.5% sodium dodecyl sulfate (SDS), and 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 1 h at 37°C. End-labeled probe 1 or 2 (10<sup>6</sup> cpm) was placed in each hybridization bag at 37°C with slight agitation overnight. The GeneScreen Plus filters were washed by the manufacturer’s instructions and placed on Kodak X-OMAT RP film overnight.

All colonies which hybridized to probe 1 during the initial screening of the SpH<sub>1</sub> library were transferred with sterile toothpicks to duplicate grid L-agar plates containing 200 μg of ampicillin per ml plus 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). Colonies which hybridized to

**Probe preparation.** Two synthetic single-stranded oligonucleotides were prepared by the Molecular Biology Resource Facility of St. Francis Hospital, using an Applied Biosystems DNA Synthesizer (model 380B). Probe 1 encoded the highly conserved redox-active site derived directly from the sequence of lpdV (Fig. 1) and was made complementary to the sense strand. When probe 1 was used as a primer, the sequence extended toward the N-terminal coding region. Probe 2 was constructed to match the sense strand encoding the N-terminal amino acids of LPD-glc (7). The N-terminal amino acid sequence of LPD-glc was translated into nucleotide sequence, using the codon preference data derived from lpdV (24). When probe 2 was used as a primer, the nucleotide sequence extended toward the active site coding region.

The probes were end labeled (20) with 20 U of T4 polynucleotide kinase, using 50 pmol of oligonucleotide and [γ<sup>32</sup>P]ATP (150 μCi; specific activity, 3,000 Ci/mmol), and the mixture was incubated at 37°C for 30 min. The labeled oligonucleotide was separated from [γ<sup>32</sup>P]ATP, using a spin Sephadex G25/50 column.
both probes were grown in 2 ml of L broth with ampicillin
overnight and rescreened with both probes using dot blots.
One of the clones isolated, pHPl, contained lpdG on a 5.3-kb insert.

Southern blots. P. putida PpG2 chromosomal DNA (2 μg)
was digested with 5 U of SphI for 2 h at 37°C. The digested samples
were electrophoresed in a 0.8% agarose gel in two
separate lanes and transferred to GeneScreen Plus mem-
brane as described above. The nylon membrane was cut in
half; one half was hybridized to labeled probe 1 and
the second half was hybridized to probe 2 at 37°C overnight
with slight agitation. The membranes were washed and placed on
film as described above, and the film was allowed to expose
for 2 days with an intensifier screen.

Determination of nucleotide sequence. The nucleotide se-
quence was determined by the chain termination method of
Sanger et al. (27). The starting materials for subcloning were
pHP1 and pHPl. pHPl was constructed by taking a 2-kb
PstI fragment of pHPl, containing the complete gene for
lpdG, and ligating it in the opposite orientation into pUC18.
The set of ordered deletions for sequencing (15) was ob-
tained by digesting the polylinker region of pHPl and pHPl
with BamHI and SacI. The former produced a site sensitive
to EcoIII, and the latter produced a site resistant to
EcoIII. The digests were then treated with S1 nuclease. The sub-
clones were ligated, transformed into E. coli TB1, and
spread onto plates containing L agar and ampicillin. Small-
scale plasmid isolation was performed on the resulting
subclones which were sized to ensure that overlapping
clones were produced in both orientations. A 7-deaza-dGTP
Reagent Kit for DNA Sequencing with Sequenase (United
States Biochemical Corp., Cleveland, Ohio) was used as
described in manufacturer’s directions to prevent compress-
sions due to high G+C content of Pseudomonas DNA.

Analysis of nucleotide sequences. The sequence data were
entered into the computer of the Oklahoma University
Genetic Computer Group. DNA sequence was analyzed
using the Genetics Computer Group’s Sequence Analysis
Software Package Version 6.0 (12) from the University of
Wisconsin Biotechnology Center which is available through
the Oklahoma University Genetic Computer Group. The
sequence was saved, edited using SEQUED, and translated
using MAP and TRANSLATE. IFASTA was used to search
amino acid data banks for homology. The N-terminal amino
acid sequence of LPD-glC (7) was used to identify the start of
the coding region. The programs ALIGN, GAP, and CON-
SENSUS were used to obtain the alignments of the amino
acid sequences. STEMLOOP and REPEAT were used to
determine whether any repeat sequences or rho-independent
terminators were present in the nucleotide sequence.

Immunodiffusion assays. Double-diffusion assays were
performed as previously described (32). The insert from
plasmid pHPl containing the complete lpdG gene was
removed by digestion with HindIII and SacI and inserted into
the same sites of the promoterless broad-host-range vector
pKT240, and the plasmid was inserted into E. coli D5Hα.
The vector designated pHPl was transferred from E. coli
DH5α to P. putida by triparental mating (13). Samples of
overnight cultures (100 μl each) of E. coli DH5α(pHP4) and
E. coli DH5α(pRK2013) were mixed with 100 μl of P. putida
JS348, and spun down and the pellet was resuspended in 50
μl of saline and transferred to a sterile filter disc and placed
on Pseudomonas Isolation Agar with 2 mg of carbenicillin
per ml. After 4 h at 37°C, the filter was suspended in 5 ml of
physiological saline and a 100-μl aliquot was plated on
the same medium. An isolated colony of P. putida JS348(pHP4)

FIG. 2. Southern blots of P. putida genomic DNA and probes 1
and 2 produce three bands. P. putida DNA was hybridized to
N-terminal probe 2 (lane A) or to probe 1 containing the redu-

dox-active site sequence (lane B). The three bands in lanes A and B at
approximately 5, 1.4, and 0.9 kb correspond to DNA fragments
encoding the three lipoamide dehydrogenases. The samples were
analyzed by electrophoresis using a series of bacteriophage λ DNA
fragments of known molecular sizes as markers (shown between the
two lanes).

was then grown in 100 ml of L broth with 2 mg of carbeni-
cillin per ml overnight at 37°C with aeration. Sondicated extracts were obtained and clarified by centrifugation at
90,000 × g.

Nucleotide sequence accession number. The GenBank ac-
cession number for the lpdG sequence is M38421.

RESULTS

Demonstration of three lipoamide dehydrogenase structural
genes in P. putida. Three bands resulted from hybridization of
P. putida genomic DNA to probe 1 containing the nucleotide sequence encoding the highly conserved redox-

active site of LPD-val (Fig. 2). The bands in the SphI digest
correspond to fragments encoding the redox-active sites of
the three lipoamide dehydrogenase structural genes in P.
putida PpG2. The largest fragment (approximately 5 kb)
contained lpdG, the medium-size fragment (approximately
1.4 kb) contained lpd3 (23a), and the smallest fragment (0.9
kb) contained part of the LPD-val gene (6). Identification of
the genes in these fragments was confirmed by restriction
enzyme analysis of their nucleotide sequences using the
MAP program.

Cloning strategy. The strategy used to isolate lpdG, the
gene encoding LPD-glC, was to use oligonucleotide probes 1
and 2 (Fig. 1) to screen genomic libraries of P. putida DNA.
A clone which reacted positively to both probes, pHPl, was
isolated. Double-strand sequencing (17) using probe 1 as a
primer identified lpdG on the basis of its N-terminal amino
acid sequence (14). Another positive colony which hybrid-
ized only to probe 1 contained an insert with the LPD-val
structural gene (6).

Nucleotide sequence of lpdG. Figure 3 shows the strategy
used to determine the nucleotide sequence of both strands of
lpdG. This procedure yielded clones which provided over-
lapping sequences in both orientations and provided the sequence of both DNA strands. The nucleotide sequence of \( \text{lpdG} \) and the deduced amino acid sequence of \( \text{LPD-glc} \) are shown in Fig. 4.

The start of the clone consists of a partial open reading frame of 168 bases encoding 56 amino acids on the same strand as \( \text{lpdG} \). This C-terminal region has 73% amino acid identity with the C-terminal region of \( E2 \) of the 2-ketoglutarate dehydrogenase from \( E. coli \) (37). There is only 30% amino acid identity with the corresponding region of \( E2 \) from branched-chain keto acid dehydrogenase of \( P. putida \) (5). Considering the above, there is a high probability that \( \text{LPD-glc} \) is the E3 component of the 2-ketoglutarate dehydrogenase operon of \( P. putida \) as are the lipoamide dehydrogenases of \( A. vinelandii \) (42) and \( P. fluorescens \) (2).

The \( E2 \) structural gene is followed by 94 bases of noncoding DNA. The \( \text{lpdG} \) coding region consists of 1,428 bases encoding 476 amino acids plus a TAA stop codon. The correct reading frame was identified with the aid of the partial amino acid sequence of \( \text{LPD-glc} \). The sequence of the 10 N-terminal amino acids of \( \text{LPD-glc} \) is Met-Thr-Gln-Lys-Phe-Asp-Val-Val-Val-Ile (7). This sequence was found in the translation beginning with the ATG at base 266 and is indicated by a single underline in Fig. 4. A likely ribosome-binding site, AGGA, precedes the initiating ATG by 7 bases and is marked SD. The highly conserved redox-active site is indicated by a double underline in Fig. 4.

The noncoding region between \( E2 \) and \( \text{lpdG} \) and the region downstream of \( \text{lpdG} \) were examined for dyad symmetry and rho-independent terminators. The REPEAT program could not identify strong repeats larger than 7 bases or rho-independent terminator. The program STEMLOOP predicted a region of dyad symmetry, following the coding region, suggesting that there is a possible stem-and-loop structure that might serve as a transcript terminator. The free energy of formation of the stem-and-loop structure which could be formed in the mRNA transcript is \(-9.4 \text{ kcal} \). The stop codon is the less common form and TAA, instead of TGA which is usually found in \( \text{Pseudomonas} \) genes (41).

**Codon usage.** The G+C content of \( \text{lpdG} \) is 60.6%, and 77% of the third codons are either G or C which is typical of organisms with a high chromosomal G+C content. In comparison, for 16 chromosomal genes of \( P. putida \) found in a computer search using Medline, the average G+C content was 62.3% and ranged from 56.6 to 66.3%.

**Amino acid composition and molecular weight.** The amino acid composition of \( \text{LPD-glc} \) predicted from the nucleotide sequence is shown in Table 2. The predicted composition agrees with that obtained by hydrolysis of \( \text{LPD-glc} \) (11). The \( M_r \) calculated from the amino acid composition in Table 2 is 49,955. When the \( M_r \) of flavin adenine dinucleotide is included, the total \( M_r \) is 50,740, which is somewhat lower than the value of 56,000 obtained by SDS-polyacrylamide gel electrophoresis (31).

**Comparison of \( \text{LPD-glc} \) to other lipoamide dehydrogenases.** The recent identifications and similarities of \( \text{LPD-glc} \) to lipoamide dehydrogenases whose structural genes have been sequenced are shown in Table 3. The percent identity of \( \text{LPD-glc} \) to the indicated lipoamide dehydrogenases is shown. Percent similarity includes identities plus percent conserved substitution i.e., substitution of a charged amino acid for a charged amino acid.

The amino acid sequence of \( \text{LPD-glc} \) has very high amino acid identity to the lipoamide dehydrogenases of \( P. fluorescens \) (2) and \( A. vinelandii \) (42). These three genes are parts of operons in which the genes immediately upstream encode the \( E2 \) component of 2-ketoglutarate dehydrogenase. In contrast, the lipoamide dehydrogenase encoded by \( \text{lpd} \) of \( E. coli \) has low homology to \( \text{LPD-glc} \) and the \( lpd \) gene is a part of the pyruvate dehydrogenase operon (37) (Table 3). It is surprising that \( \text{LPD-glc} \) has comparatively low identity to \( \text{LPD-val} \), considering that these lipoamide dehydrogenases are from the same organism.

All lipoamide dehydrogenases whose sequences have been reported have the same domain structure as glutathione reductase, whose structure was solved by X-ray crystallography (18). Glutathione reductase is composed of four domains, the flavin adenine dinucleotide and NADP + binding domains and central and interface domains (40). The computer programs ALIGN, GAP, and CONSENSUS were used to align the sequence of \( \text{LPD-glc} \) with that of glutathione reductase (data not shown), which confirmed that \( \text{LPD-glc} \) contains the four domains of glutathione reductase.

**Separate expression of \( \text{LPD-glc} \).** \( E. coli(pHP1) \) and \( E. coli(pHP3) \) both expressed \( \text{lpdG} \) even though the \( P. putida \) DNA insert is in the orientation opposite that of the lac promoter in pHP1 (Table 1). This suggests that the 94-base noncoding region between \( E2 \) and \( E3 \) might contain a promoter for separate expression of \( \text{lpdG} \), as is the case with \( lpd \) of \( E. coli \) (34). Plasmid pHP4 contains an insert which includes the C-terminal portion of the \( E2 \) structural gene plus the noncoding intergenic region and also contains the entire structural gene of \( \text{lpdG} \) in pKT240 (see Materials and Methods). pHP4 was inserted into \( P. putida \) JS348, a \( \text{lpdG} \) mutant (31), by triparental mating. An immunodiffusion assay was performed to detect the presence of \( \text{LPD-glc} \) in extracts of \( P. putida \) JS348(pHP4) and \( P. putida \) JS348(KT240) grown in L broth with carbenicillin (Fig. 5). The lines of immunoprecipitation clearly show expression of \( \text{lpdG} \) from pHP4. Since pKT240 does not contain a promoter, \( P. putida \) JS348(pHP4) must be using the noncoding region upstream of \( \text{lpdG} \) as a promoter. This result verifies that the lipoamide dehydroge-
FIG. 4. Nucleotide sequence of pH3 and deduced amino acid sequences. Bases 1 to 171 encode the C-terminal region of the presumed E2 (transsuccinylase) component. The underlined amino acids are the N-terminal amino acids of LPD-glc (7). The redox-active disulfide region is indicated by a double underline. A possible stem-and-loop terminator structure is indicated by opposing arrows. The underlined sequence marked SD is the Shine-Dalgarno ribosome-binding site. The termination codon (***+) is also shown.
TABLE 2. Amino acid composition of LPD-glc predicted from nucleotide sequence and amino acid hydrolysis

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<td>Methionine</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Isoleucine</td>
<td>13</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>35</td>
<td>38</td>
<td></td>
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<tr>
<td>Tyrosine</td>
<td>11</td>
<td>8</td>
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<tr>
<td>Phenylalanine</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Lysine</td>
<td>35</td>
<td>33</td>
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<tr>
<td>Histidine</td>
<td>8</td>
<td>9</td>
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</tr>
<tr>
<td>Arginine</td>
<td>13</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>4</td>
<td>4</td>
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</table>

* The original data were recalculated on the basis of M = 50,000 (11).

nase can be expressed separately from the rest of the operon.

DISCUSSION

Previous biochemical studies (7) and now genetic analysis confirms the presence of three lipoamide dehydrogenases in P. putida. Southern blots of P. putida PpG2 genomic DNA digested with SphI hybridized to probes matching the coding sequences of the redox-active site and the N-terminal regions of LPD-glc. Probe 1 containing the sequence encoding the redox-active site hybridized to P. putida genomic DNA digested with SphI, producing three bands corresponding to the active site of the three lpd genes. Identification of the three genes was verified by restriction mapping of the nucleotide sequences and restriction mapping of the clones. Probe 2 also hybridized to the fragments, but it hybridized with lower stringency to the 5- and 1.4-kb fragments and hybridized faintly to the 0.9-kb fragment. Since LPD-glc and LPD-3 have similar N-terminal amino acids, this was expected, but it was somewhat surprising that probe 2 also hybridized, even weakly, to the LPD-val gene fragment.

pHP1 contains 56 codons of a reading frame which encodes the C-terminal region of an E2 keto acid dehydrogenase component. This E2 component has 73% amino acid identity with the C-terminal region of 2-ketoglutarate dehydrogenase E2 component of E. coli but only 25% identity with E2 of E. coli pyruvate dehydrogenase (36). It appears that lpdG is a part of the 2-ketoglutarate dehydrogenase operon as are the structural genes for lipoamide dehydrogenases of A. vinelandii (42) and P. fluorescens (2).

In P. putida, P. fluorescens and A. vinelandii lipoamide dehydrogenase structural genes are a part of the 2-ketoglutarate dehydrogenase operon. In E. coli, lpd is attached to the pyruvate dehydrogenase operon. In all three cases, it is likely that these genes encode lipoamide dehydrogenase for pyruvate and 2-ketoglutarate dehydrogenases. In E. coli, lpd can be expressed independently of the rest of the operon from a promoter 200 bp upstream from the start of lpd (35, 37). The lpd gene of A. vinelandii was strongly expressed in E. coli even though the insert was cloned in the orientation opposite that of the lacZ promoter, which also suggests independent expression of this gene (42). Independent expression was not detected from the 69-bp intracistronic region between E2 and E3 of P. fluorescens (2). We have shown in this study that lpdG can be expressed independently of the 2-ketoglutarate dehydrogenase operon, and it seems likely that there is a lpdG promoter in the 94 bases of noncoding DNA upstream of lpdG.

The extremely high amino acid sequence identity of LPD-glc with lipoamide dehydrogenases from A. vinelandii and P. fluorescens (Table 3) is interesting and suggests that these three proteins are closely related evolutionarily. It will be interesting to see whether the homology between the corresponding E1 and E2 components will be as high. Recently, the structural genes for the E1 and E2 components of A. vinelandii 2-ketoglutarate dehydrogenase were cloned and their nucleotide sequences were determined (28, 43). For comparison, there is 59 and 63% amino acid identity between these proteins and the E1 and E2 components of the 2-ketoglutarate dehydrogenase of E. coli (10, 34).

TABLE 3. Percent amino acid identities and similarities of LPD-glc with other lipoamide dehydrogenases

<table>
<thead>
<tr>
<th>Lipoamide dehydrogenase</th>
<th>% Identity</th>
<th>% Similarity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. fluorescens</td>
<td>81.8</td>
<td>89.9</td>
<td>2</td>
</tr>
<tr>
<td>A. vinelandii</td>
<td>79.5</td>
<td>87.2</td>
<td>42</td>
</tr>
<tr>
<td>Pig</td>
<td>48.7</td>
<td>67.9</td>
<td>23</td>
</tr>
<tr>
<td>Human</td>
<td>45.9</td>
<td>67.7</td>
<td>23</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>45.6</td>
<td>67.9</td>
<td>26</td>
</tr>
<tr>
<td>E. coli</td>
<td>39.9</td>
<td>62.7</td>
<td>37</td>
</tr>
<tr>
<td>P. putida LPD-val</td>
<td>37.7</td>
<td>61.7</td>
<td>6</td>
</tr>
</tbody>
</table>

FIG. 5. Expression of lpdG independently of the rest of the operon. Wells: 1, 300 µg of protein from P. putida JS348(pHP4); 2, 2 µg of purified LPD-glc; 3, 300 µg of protein from P. putida JS348(pKT240); cw, 20 µl of a 1:1 dilution of anti-LPD-glc.

ACKNOWLEDGMENT

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