Pseudomonas cepacia 2,2-Dialkylglycine Decarboxylase

SEQUENCE AND EXPRESSION IN ESCHERICHIA COLI OF STRUCTURAL AND REPRESSOR GENES*

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A 3969-base pair PstI-PstI fragment of Pseudomonas cepacia DNA containing the gene for the pyridoxal 5'-phosphate dependent 2,2-dialkylglycine decarboxylase (pyruvate) (EC 4.1.1.64) was cloned in Escherichia coli. The insert was sequenced by the dideoxy method using nested deletions from both ends, revealing a central 1302-base pair region that codes for the decarboxylase subunit. The recombinant enzyme was expressed in E. coli, purified to homogeneity, and sequenced at the amino terminus. Also, a cofactor-labeled active site peptide was sequenced. The carboxyl terminus of the deduced amino acid sequence is homologous with the carboxyl terminus of mammalian ornithine aminotransferase; the active site sequence is similar to the active site sequences of several other aminotransferases. No homologies with known decarboxylase sequences could be found. Expression of the decarboxylase gene is negatively controlled by a 687-nucleotide sequence upstream of and diverging from the structural gene. Expression is induced by S-isovaline, 2-methylalanine, and D-2-aminobutanoic acid, but not by glycine, D- or L-alanine, L-2-aminobutanoic acid, R-isovaline, or other alkyl amino acids.

The 2,2-dialkylglycine decarboxylase of the soil bacterium Pseudomonas cepacia was first reported by Aaslestad and Larson (1964) and was later investigated in several laboratories (Bailey and Dempsey, 1967; Bailey et al., 1970; Lamartiniere et al., 1971; Honma et al., 1972; Sato et al., 1978; and Keller and O'Leary, 1979). This pyridoxal 5'-phosphate-dependent enzyme catalyzes decomposition of substrate amino acids such as 2-methylalanine and isovaline in two steps: (i) carboxylate replacement reaction (Sukhareva, 1986). The dialkylglycine decarboxylase is of interest because it normally catalyzes both decarboxylation and amino transfer. Therefore, the question arises whether this enzyme is an aminotransferase that through evolution has added a decarboxylase capability or is a decarboxylase that has evolved an amino transfer capability. We provide a preliminary answer to this question by showing that the dialkylglycine decarboxylase primary structure is homologous to several aminotransferases but not to decarboxylases.

The biological role of the dialkylglycine decarboxylase remains unclear. The substrates 2-methylalanine and isovaline occur naturally as major constituents of cytotoxic peptides produced by soil fungi such as Trichoderma viride (Bruckner et al., 1980; Bruckner and Pryzbylski, 1984; Schmitt and Jung, 1985) and as organic components of carbonaceous meteorites (Kvenvolden et al., 1971). Racemic isovaline and 2-methylalanine have been shown recently in an iridium-rich Cretaceous-Tertiary boundary layer, further supporting an extraterrestrial source for this material (Zhao and Bada, 1989). Thus, the enzyme may have evolved to use the rare dialkylglycines of cosmic origin, or it may be a part of a metabolic pathway for breaking down cytotoxic peptides and the constituent amino acids.

The available structural information about the 2,2-dialkylglycine decarboxylase is sparse. Lamartiniere et al. (1971) showed by equilibrium sedimentation that a dialkylglycine decarboxylase isolated from P. cepacia has a molecular mass of 188 kDa with four identical 47-kDa subunits. They also reported a peptide map and amino acid composition data consistent with a 47-kDa subunit. Sato et al. (1978) also studied the P. cepacia dialkylglycine decarboxylase, showing by gel electrophoresis that the 180-kDa holoenzyme contained four identical subunits of approximately 45 kDa and presenting chemical labeling evidence for a catalytically important histidine residue.

We undertook the cloning and sequencing of the structural gene of the P. cepacia 2,2-dialkylglycine decarboxylase to determine whether this enzyme is structurally and evolutionarily more closely related to the B6-dependent decarboxylases than to aminotransferases. We report here (i) the release of carbon dioxide and a ketone with transfer of the amino group to the cofactor to give enzyme-bound pyridoxamine 5'-phosphate and (ii) amino transfer from cofactor to pyruvate forming L-alanine and regenerating the cofactor in the aldehyde oxidation state. The decarboxylation step is analogous to the so-called abortive decarboxylation catalyzed by several pyridoxal 5'-phosphate-dependent amino acid decarboxylases, which competes with the normal hydrogen for carboxylate replacement reaction (Sukhareva, 1986). The dialkylglycine decarboxylase is of interest because it normally catalyzes both decarboxylation and amino transfer. Therefore, the question arises whether this enzyme is an aminotransferase that through evolution has added a decarboxylase capability or is a decarboxylase that has evolved an amino transfer capability. We provide a preliminary answer to this question by showing that the dialkylglycine decarboxylase primary structure is homologous to several aminotransferases but not to decarboxylases.

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sequence of a cloned 3969-bp segment of \( P. \) cepacia DNA containing the 2,2-dialkylglycine decarboxylase structural gene, (ii) purification of the recombinant decarboxylase, (iii) determination of the amino acid sequence of the amino terminus and the active site peptide, and (iv) alignment of the deduced amino acid sequence of the deduced amino acid sequence of this decarboxylase with various aminotransferases.

Asselsted and Larson (1964) found that \( P. \) cepacia produced the decarboxylase only when the organism's minimal salt-glucose growth medium was supplemented with 2-methylalanine. This suggested that decarboxylase gene expression is induced in some way by the dialkylglycine substrate. We have re-examined this question using the cloned and sequenced \( P. \) cepacia DNA. We report here that the cloned DNA codes for an intact dialkylglycine decarboxylase repression-induction system that functions in \( Escherichia \) coli. Functional analysis of partially deleted plasmids and computer analysis of the sequence upstream of the structural gene provide evidence for a repressor gene. Also, several 2,2-dialkylglycine stereoisomers were synthesized and tested as inducers of decarboxylase gene expression.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

**Cloning**—Isolation of the dialkylglycine decarboxylase gene was simplified by the inability of \( E. \) coli to metabolize 2,2-dialkylglycines. Thus, a library created by ligating \( PstI \)-restricted \( P. \) cepacia DNA into the pBR322 \( PstI \) site was screened for the dialkylglycine decarboxylase gene by plating the transformation mixture on LB/tetacycline agar to select transformants and subsequently making a replica transfer to 2-methylalanine/glucose agar. Isolated from one of several colonies that survived the transfer was a 16-kbp recombinant plasmid containing several heterologous \( PstI \) fragments. A \( PstI \) digest of the plasmid was subcloned back into \( PstI \)-cut pBR322 and a pair of smaller recombinant plasmids, pKBD6 and pKBD14, were isolated; these were shown by restriction analysis to differ only in the orientation of a 4.0-kbp insert (Fig. 1). The 4.0-kbp \( PstI-PstI \) fragment was transferred into pUC19 to give pUC19C7 and the slightly larger XbaI-EcoRI fragment of pUC19C7 (sequencing later showed that the EcoRI site is 77 bases away from the end of the insert) was transferred into pGEM-7Z14 (+) to give pGEM-7Z14 (Fig. 1). Also, pUC19H1 was constructed from pUC19C7 by deletion of a 1.3-kbp SphI-SphI fragment (not shown). All the above recombinant plasmids, pKBD6, pKBD14, pUC19C7, and pGEM-7Z14, confer on \( E. \) coli the ability to grow on 2-methylalanine/glucose agar.

**DNA Sequence**—Sequencing was carried out using a modified Sanger dideoxy method (Sanger et al., 1977; Kraft et al., 1988). The + strand of the insert (the upper strand in Fig. 1 and lower strand in Fig. 2; the coding strand for the decarboxylase structural gene) was sequenced using a 20-mer primer complementary to pGEM-7Z sequences on the left side of the insert and, as templates, plasmids with progressively larger deletions from the left. The – strand of the insert (the lower strand in Fig. 1 and upper strand in Fig. 2; the coding strand for the putative repressor gene) was sequenced using a 20-mer primer complementary to pUC19 sequences on the right side of the insert and, as templates, plasmids with progressively larger deletions from the right. One hundred percent of the control and structural genes was sequenced on both strands; 90% of the remaining sequence was determined on both strands.

**Enzyme Expression and Purification**—The recombinant decarboxylase was purified from \( E. \) coli JM109 carrying pGEM-7Z14/8b, a truncated derivative of pGEM-7Z14 with 1332 base pairs deleted from the left end leaving 63 nucleotides ahead of the decarboxylase gene. In this and other pGEM-7Z14 derivatives, the vector lac promoter lies upstream of and points toward the dialkylglycine decarboxylase gene (Fig. 1). When carbencillin and IPTG were added to the JM109/pGEM-7Z14/8b growth medium, the dialkylglycine decarboxylase comprised about 0.5% of the cell extract, similar to

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1 The abbreviations used are bp, base pair(s); kb, kilobase pair(s); IPTG, isopropyl-1-thio-β-D-galactopyranoside; MOPS, 3-(N-morpholino)propanesulfonic acid; PLP, pyridoxal 5'-phosphate; SDS, sodium dodecyl sulfate; FPLC, fast protein liquid chromatography; AU, absorbance units.

2 Portions of this paper (including "Experimental Procedures," part of "Results," Tables 1 and 2, and Figs. 5, 6, and 9) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
levels induced by 2-methylalanine in *Pseudomonas* (Keller and O'Leary, 1978; Sato et al., 1978). This decarboxylase was purified from JM109/pGEM7Z14/8b in three steps using ammonium sulfate precipitation, ion exchange chromatography, and FPLC ion exchange chromatography. The purified enzyme was homogeneous as judged by SDS polyacrylamide gel electrophoresis (see Table 1 and Fig. 5, Miniprint).

**Sequence of the Amino Terminus**—The purified protein was sequenced at the amino terminus by automated Edman degradation. The results are included in Fig. 3. In the 14 cycles in which the phenylthiohydantoin-amino acid yields were high enough to make clear identifications, the experimentally determined residues matched the predicted ones. This sequence also shows that the decarboxylase terminal N-formylmethionine has been removed, but no additional amino-terminal proteolysis has occurred.

**Sequence of the Active Site Peptide**—The decarboxylase active site was labeled using a variation of the method first introduced by Fischer et al. (1958). In this procedure, the active site lysine-[4-14C]pyridoxal 5'-phosphate imine bond of the holoenzyme was reduced with sodium borohydride. The labeled active site peptide was isolated by trypsin digestion followed by reversed phase high performance liquid chromatography separation of the resulting peptides. The major radioactive peptide was sequenced, and the amount of radioactivity released by each cycle of the automated sequencer was determined. Sixteen cycles were clearly identifiable: all except cycle 12 agreed with the predicted amino acid sequence beginning after R260. Cycle 12 produced the most radioactivity and showed no identifiable peak on the Sequencer (see Miniprint for Fig. 6). This is presumably the cofactor-labeled lysine residue predicted by the DNA sequence.

**Identification of the Dialkylglycine Decarboxylase Structural Gene**—The ATG initiation codon of the 1302-base dialkylglycine decarboxylase structural gene is at position 1395 of the + strand of the insert (Figs. 1-3). This ATG marks the beginning of the only large reading frame within the region of the insert that is necessary for expression of decarboxylase activity. The predicted amino acid sequence of this coding region matches the experimentally determined sequences at the amino terminus and active site.

A CCGGAG sequence was found ahead of the structural gene that is similar to the ribosome binding sites ahead of other bacterial genes (Min et al., 1988; Stormo et al., 1982). Also, 40 nucleotides downstream of the TAA stop codon is a 31-base GC-rich sequence with dyad symmetry that could form a 12-base stem, 7-base loop structure. The sequence immediately downstream of this dyad is not T-rich; thus, this sequence is similar to ρ-dependent transcription terminators in other bacterial operons (Platt, 1986).

**Codon usage within the decarboxylase coding region** is strongly biased toward codons with G or C in the third position: 401 out of 434 codons (92%) have G or C in the third position (Table 2, Miniprint). This percentage is consistent with the prediction of Bibb et al. (1984) that 88% of the third positions will have G or C in a gene having 68% G+C. Codon bias analysis was particularly helpful in identifying the reading frame of the decarboxylase structural gene in this GC-rich DNA, since the noncoding reading frames are not nearly
as biased toward G and C in the third position.

The length and amino acid content of the decarboxylase polypeptide deduced from the nucleotide sequence is nearly identical with that determined by Lamartiniere et al. (1971) for the dialkylglycine decarboxylase from another Pseudomonas isolate. A sequence identity of 83% is predicted for these two proteins using Cornish-Bowden's method based on amino acid content (Cornish-Bowden, 1979). Considering the possibility of error in the amino acid determination, it is likely that the enzyme studied in this work, which is the same one studied by Sato et al. (1978), is identical with the one studied by Lamartiniere et al. (1971).

Homology Searches—The deduced amino acid sequence of the P. cepacia dialkylglycine decarboxylase was compared with the Protein Identification Resource database (Release 17) and with translations of all six reading frames of each DNA sequence in the GenBank (Release 60) and the EMBL (Release 15) databases (Henikoff and Wallace, 1988). The GenBank search located three sequences that show significant homology to the dialkylglycine decarboxylase: the 423-amino acid translation of yeast ornithine aminotransferase mRNA (Degols, 1987), and the 439 amino acid translations of rat (Mueckler and Pitot, 1985) and human (Mitchell et al., 1988) ornithine aminotransferase mRNAs. Comparison of these sequences with the deduced dialkyglycine deacryboxylase sequence using a moving 30-amino acid window (Wilbur and Lipman, 1983) gives log odds scores of 10.5, indicating a significant sequence similarity (Henikoff and Wallace, 1988). No decarboxylase sequence scored higher than 9.8 in the search procedure and alignments of several decarboxylase sequences with the dialkylglycine deacryboxylase sequence showed no noteworthy similarities. Homologies with other aminotranfersases were weak except near active site lysines as discussed below. The E. coli branched chain aminotransferase (Inoue et al., 1988) and phosphoserine aminotransferase (Visali et al., 1989) showed no homology with the dialkylglycine decarboxylase or ornithine aminotransferase even in active site regions.

Dialkylglycine Decarboxylase Gene Induction—The kinetics of decarboxylase induction in E. coli host DH5a carrying plasmid pKBD6 were studied with several amino acids that are substrates for the dialkylglycine decarboxylase and that therefore might be expected to be gene inducers. These are racemic isovaline, 2-methylalanine, and D-alanine, which are decarboxylated by the enzyme, and L-alanine, which is transaminated (Bailey et al., 1970). As shown in Fig. 7, when DH5a/pKBD6 was grown in minimal media containing ammonium chloride as nitrogen source, decarboxylase specific activity was low. Addition of either 2-methylalanine or racemic isovaline caused an immediate and rapid increase in decarboxylase specific activity, approximately paralleling growth. Decarboxylase production ceased with growth and remained stable for several hours in the induced cells (data not shown). In contrast, when D- or L-alanine was added to growing cell cultures, no decarboxylase production ensued.

Induction Stereochemistry—The induction phenomenon was further investigated by surveying the inducing ability of the separate isovaline stereoisomers and other structurally similar amino acids (Table 3). JM109/pGEM7Z14 was grown overnight in YT-ampicillin plus 20 mM amino acid, then cell sonicate supernatants were assayed for protein and dialkylglycine decarboxylase activity. The assays showed decarboxylase specific activities 10 times the untreated control only if S-isovaline, 2-methylalanine, or L-2-amino butanoic acid had been added to the culture medium; 1 aminoacylpeptanecarboxylic acid induced an intermediate level. The other amino acids tested induced no better than the culture medium itself.

The role of the upstream DNA in controlling decarboxylase gene expression was investigated by determining decarboxylase levels in E. coli JM109 carrying pGEM-7Z14 or one of 10 plasmids derived from it with various lengths of insert removed by exonuclease treatment. In these constructs, transcription from the vector lac promoter, which is upstream of the insert and pointing toward it, was controlled by maintaining the plasmids in host strain JM109, an overproducer of the lac repressor (Yanisch-Perron et al., 1985). Fig. 8 shows decarboxylase specific activities of the various JM109 strains grown in YT, YT plus 2-methylalanine, or YT plus IPTG.

Most obviously, truncation by 1440 or 1651 bp completely prevented production of active dialkylglycine decarboxylase under all growth conditions. These deletions removed part of the decarboxylase structural gene that has been shown by sequencing to begin at 1395 of the insert. In the presence of IPTG, the strains carrying the next two larger plasmids (1332 and 1314 bp removed) produced high levels of enzyme, about 500-fold over background. When grown in untreated or 2-methylalanine-treated medium, these latter strains produced decarboxylase levels about 300-fold over background. Strains carrying the next three larger plasmids (1012, 907, and 77 bp removed from the original insert), 2-methylalanine-treated levels, while the effect of IPTG decreased to nil.

Fig. 7. Induction of dialkylglycine decarboxylase activity in E. coli DH5a/pKBD6 cultures by various amino acids. One-liter cultures containing minimal salts, glucose, NaCl, and tetracycline (12.1 g/ml) were supplemented at the indicated time with amino acids (final concentration, 10 mM). Absorbance of the culture was measured at 600 nm in a 1-cm cell (— — —). Each activity assay contained 60 absorbance units of resuspended bacteria (— — —).
**TABLE 3**

| Amino acid                  | pro-R | pro-S | Specific activity (nmoI h⁻¹ mg⁻¹) | S.D. (No. of experiments) |
|-----------------------------|-------|-------|---------------------------------|--------------------------|
| Control                     |       |       | 5.12                           | 4.01 (14)                |
| Glycine                     | H     | H     | 5.20                           | 0.20 (2)                 |
| L-Alanine                   | H     | CH₃   | 1.74                           | 0.20 (2)                 |
| L-2-Aminobutanoic acid      | H     | CH(CH₂)₂ | 48.8  | 12.0 (4)                  |
| L-Norvaline                 | H     | CH(CH₂)₂ | 4.31   | 0.08 (2)                 |
| L-Valine                    | H     | CH(CH₂)₂ | 4.22   | 0.16 (2)                 |
| L-Isolucine                 | H     | CH(CH₃)CH(CH₂) | 4.05   | 0.29 (2)                 |
| D-Alanine                   | CH₃   | H     | 3.76                           | 0.20 (2)                 |
| D-2-Aminobutanoic acid      | CH₂CH₃ | H     | 2.56                           | 0.08 (2)                 |
| D-Norvaline                 | CH₂CH₂CH₃ | H     | 4.21                           | 0.08 (2)                 |
| D-Valine                    | (CH₂)₃CH | H     | 4.09                           | 0.25 (2)                 |
| D-Isolucine                 | CH₂CH₂CH(CH₂) | H     | 3.39                           | 0.12 (2)                 |
| 2-Methylalanine             | CH₂CH₃ | CH₃   | 58.7⁰                           | 22.7 (16)               |
| S-Isolucine                 | CH₂CH₃ | CH₃   | 41.8⁰                           | 11.1 (4)                 |
| S-2-Methylnorvaline         | CH₂CH₃ | CH₂CH₂CH₃ | 12.8  | 5.95 (4)                 |
| R-Isolucine                 | CH₂CH₂CH₃ | CH₃   | 6.45                           | 1.45 (4)                 |
| R-2-Methylnorvaline         | CH₂CH₂CH₃ | CH₃   | 5.45                           | 1.20 (4)                 |
| 1-Aminocyclopentane carboxylic acid | CH₂CH₃ | CH₂CH₃ | 16.5⁰                          | 10.7 (4)                 |

* For duplicate experiments (n = 2), the average deviation is given.
* Greater than control at the 99% confidence level by the Mann-Whitney test.

A surprising finding was the lack of an active site histidyl-lsine pair in the 2,2-dialkylglycine decarboxylase. All known procaryotic pyridoxal phosphate-dependent decarboxylases, including E. coli arginine, lysine, ornithine, and glutamate decarboxylases (Tanase et al., 1979) and two eucaryotic decarboxylases, the pig kidney L-3,4-dihydroxyphenylalanine decarboxylase (Tanase et al., 1979) and Morganella morganii histidine decarboxylase (Hayashi et al., 1986) have such a sequence at the active site. In the M. morganii histidine decarboxylase site-directed mutagenesis studies suggest a hydrogen-bonding role for the histidyl next to the active site lysine that can be partially assumed by glutamine (Vaaler and Snell, 1989). Sato et al. (1978) found that diethyl pyrocarbon-
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...ate modifies one of seven histidines of the dialkylglycine decarboxylase, inhibiting the decarboxylation reaction selectively. Thus, this enzyme probably still requires the histidine catalyst, but it is elsewhere in the active site instead of adjacent to lysine.

Maximum levels of dialkylglycine decarboxylase expression from these plasmids in E. coli was similar to that in 2-methylalanine-induced P. cepacia (Keller and O'Leary, 1979; Lamartiniere et al., 1971). Steps were taken to maximize expression of the cloned decarboxylase gene by orienting the gene downstream of the strong inducible lac promoter and removing (in pGEM-7Z14/8b) all but 63 bp of cloned DNA upstream of the structural gene. We ascribe the low expression levels to translational barriers. Slow translation could be caused by several arginine codons, namely CGA, AGG, and CCG that are associated with genes weakly expressed in E. coli (Bulner, 1988). These codons occur, respectively, once, twice, and six times in the decarboxylase structural gene. Another translational barrier could be a ribosome binding sequence that is nonoptimal for E. coli. Recent studies have shown that in E. coli the mRNA ribosome binding site occupies positions -13 to -8 relative to the initiation codon and has a consensus sequence of AAGGAG (Min et al., 1988; Stornm et al., 1982), whereas that site ahead of the dialkylglycine decarboxylase gene has the sequence CCGGAG.

Control of decarboxylase gene expression is probably exercised by an upstream repressor gene. The putative repressor gene was located by decarboxylase gene induction experiments with strains carrying plasmids with the Pseudomonas DNA insert truncated by various amounts (Fig. 8). pGEM-7Z14 and the next three smaller plasmids (from 77 to 447 bp of the insert removed) all show complete repression of decarboxylase gene expression in the absence of 2-methylalanine and complete de-repression by 2-methylalanine. Thus, the control system is intact and identical in these four plasmids. Truncation by another 211 bp (656 bp total) results in a dramatic lowering of decarboxylase expression to the background level even in the presence of 2-methylalanine. This 658-bp shortening removes 10 codons from the 3' end of the repressor coding region, with the removed DNA probably encoding all or part of a 2-methylalanine-binding domain at the repressor's carboxyl terminus. The portion of the gene coding for the repressor's DNA binding domain is left untouched in this plasmid and is likely still transcribed from a promoter at the other end. The resulting shortened or modified protein could still bind to an operator sequence, but would no longer be affected by 2-methylalanine. Truncation by 907 or 1012 bp results in partial repression of decarboxylase expression, which probably is due to synthesis of portions of the DNA binding domain that retain some affinity for the operator site. Finally, removal of 1314 or 1332 bp abolishes all repression by removing all or most of the DNA coding for the DNA binding domain, but still leaves a promoter on both plasmids just ahead of the decarboxylase gene. Expression levels from these latter plasmids are identical with the 2-methylalanine-induced levels observed with the larger plasmids, as expected if RNA polymerase had unrestricted access to the Pseudomonas dialkylglycine decarboxylase promoter. While the results indicate that the decarboxylase promoter is within 75 nucleotides of the structural gene, its precise location and sequence have not been established; there are no clear sequence homologies with known Pseudomonas or E. coli promoters.

IPTG-induced decarboxylase gene expression was greater the closer the lac promoter was to the decarboxylase gene (Fig. 8). In pGEM-7Z14 and the next smallest plasmid, transcription from the lac promoter is weak enough that it can be completely blocked by the dialkylglycine decarboxylase repressor. As the intervening DNA is shortened, which may remove transcription-terminating sequences, repressor binding only partially blocks read-through from the lac promoter. Expression was highest when the lac-decarboxylase separation was 600 bp or fewer.

The most likely candidate for the repressor gene is a 687-nucleotide region beginning 81 bases upstream from the decarboxylase structural gene and coding for a 229-amino acid protein (Figs. 1 and 4). This coding region extends 30 bp past the truncation site that destroys the repressor's sensitivity to 2-methylalanine (Fig. 8). The gene product is predicted to be basic, containing 90% arginine plus lysine. This composition is similar to the eucaryotic histones H3 and H4. Just ahead of this reading frame (−14 to −6 relative to its ATG start codon) is a purine-rich region AAGGAAUAG that could function as a ribosome binding site for translation of the repressor mRNA. No significant sequence similarities between the amino acid sequence predicted for this repressor and any of the translated reading frames of the GenBank DNA sequence (Henikoff and Wallace, 1988) could be found.

The stereochemical characteristics of the amino acid binding site responsible for modulating DNA binding are apparent in Table 3. These data suggest that the amino acid binding domain of the repressor incorporates sites for each a-alkyl group, besides ionic sites for the a-ND2+ and/or the a-COO−. One alkyl group binding site, the pro-R one, interacts with a pro-R methyl of the substrate, but is too small to accept larger alkyl groups. The other site, the pro-S one, binds either a pro-S methyl or ethyl, but nothing larger. Additionally, since neither glycine nor D- or L-alanine induce, an inducer of this gene must contain at least two methylene groups on the a substituents; for example, two methyls as in 2-methylalanine or an ethyl on one side and a hydrogen on the other side as in 2-amino-2-methylalanine. These groups would provide a minimum hydrophobic interaction energy with the repressor binding site. Only 2-methylalanine, S-isovaline, and 1-2-amino-2-methylalanine satisfy these criteria. 1-Aminocyclopentanecarboxylic acid is a weak inducer, perhaps fitting portions of its ring β-methylene into both alkyl group binding sites.

The stereospecificity of dialkylglycine decarboxylase gene induction by isovaline is opposite that of the decarboxylase. Aastsetad et al. (1986) reported that the Michaelis constants of the R and S isomers are 1.0 mM and 25 mM, respectively. And relative Vmax/Km values, which correlate with affinity of the enzyme for the transition state of the decarboxylation reaction are 7.9 and 1.0 for R and S isomers, respectively. Sterically, the enzyme is more flexible than the repressor; it decarboxylates several amino acids that do not induce decarboxylase gene expression, including D-alanine (Bailey et al., 1970), racemic 2-methylbutanolic acid (Tabara et al., 1969), and 1-amino-4-cyclopentanecarboxylic acid.3 Also, several other amino acids are decarboxylate substrates but have not been tested for gene induction, including 1-aminocyclobutanecarboxylic acid, 2 amino 2 ethylbutanoic acid, and racemic 2-methylserine.

Another surprising stereochemical consequence of these results is that the only known biological sources of isovaline, the so-called peptaibol antibiotics of soil fungi, contain R-isovaline and not S-isovaline (Boech et al., 1982). Thus it is unlikely that the dialkylglycine decarboxylase genes studied here have evolved specifically to metabolize the isovaline occurring in peptide antibiotics. These genes and gene products more likely evolved to metabolize 2-methylalanine, which

3 J. W. Keller, unpublished results.
is achiral and is present in greater amounts than isovaline in the peptaibol antibiotics (Schmitt and Jung, 1985).

Our results cannot be explained by operation of a positive control system, since decarboxylase expression returns to derepressed levels once all or most of the control gene has been deleted. Nor is it likely that decarboxylase gene expression is controlled in trans from E. coli DNA, since (i) the host cannot metabolize dialkylglycines and therefore probably does not have receptors that bind both the dialkylglycines and certain sequences of exogenous DNA and (ii) de-repression is stereospecific and consistent, making the action of a nonspecific DNA binding protein unlikely. Control is not exercised by the DNA binding protein unlikely. Control is not exercised by gene, since plasmids truncated in that region still show the substituted amino acids. While the function of this system turns on decarboxylase gene expression, rather than a multi-

repressor that is regulated by three closely related alkyl-
glycine sequences suggests that a unique protein structure is involved. We have also shown that the P. cepacia dialkylgly-
cide decarboxylase has a unique structure that is not homol-
gous to known amino acid decarboxylase sequences but is instead closely related to the eucaryotic ornithine aminotransferases and other aminotransferases. Thus, it may be more properly described as a decarboxylating aminotransferase rather than an aminotransferring decarboxylase. It is likely that an in vivo function of this enzyme in Pseudomonas is to decarboxylate dialkylglycines since enzyme production in Pseudomonas is stimulated by 2-methylalanine (Aaslestad and Larson, 1964). Further studies of the biological function, mechanism, and structure of this decarboxylating amino transferase are underway.

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Continued on next page.
EXPERIMENTAL PROCEDURES

Strains and Media. For transformations with plasmid DNA, the E. coli strain used was W3110, which carries the lacZ gene on pBR322. E. coli (HB101, λlacZ58, lacZΔM15) was used for overproduction of L-2,3-diaminopropionate (DAP) synthetase and in the construction of lac operons. LB medium or plates were used for E. coli growth, and minimal medium or plates were used for B. subtilis growth. For B. subtilis transformations, the medium used was DAP minimal medium or plates (DAP-M) (Johnson et al., 1986). B. subtilis strains were grown in liquid minimal medium or plates (DAP-M) or on minimal DAP-M plates supplemented with 100 μg/ml of erythromycin. All experiments were performed at 30°C.

Enzyme Assays. The precision and reproducibility of the procedures for the determination of the various enzymatic activities were adequate to allow the detection of the differences observed between the substrate specificities of the various enzymes. The enzymes were assayed as described (Harris and Young, 1986). The activities were determined with one unit defined as the amount of enzyme that catalyzes the formation or consumption of 1 μmol of product per minute. All assays were performed in triplicate.

RESULTS

Table 1: Purification of Recombinant 2,2-Dialkylglycine Decarboxylase

| Protein (mg) | Total U | U/mg protein | Yield (%G) |
|-------------|---------|--------------|-----------|
| Cell Extract | 260 | 770 | 100 |
| DEAE-Sephacel | 0.281 | 340 | 39 |
| Mono Q | 0.126 | 36 | 24 |

*Data represent the average of triplicate determinations. The yield was calculated as the percentage of activity recovered from the cell extract.

DISCUSSION

The results presented in this study suggest that the 2,2-dialkylglycine decarboxylase from B. subtilis 168 is a membrane-bound enzyme that is not solubilized by detergent extraction. The enzyme activity was detected in the cell extract, and the specific activity was significantly higher than that obtained from the crude cell extract. The enzyme was purified to homogeneity by affinity chromatography on a column of Mono Q, and the purified enzyme was stable for at least 1 week at 4°C. The purified enzyme had a specific activity of 240 U/mg, which is significantly higher than that obtained from the crude cell extract. The enzyme was stable for at least 1 week at 4°C. The enzyme was stable for at least 1 week at 4°C. The enzyme was stable for at least 1 week at 4°C.

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Fig. 6. Radioactivity released with each sequencing cycle during sequencing of the 7H-pyridofuran 5'-phosphothioate-labeled tryptic peptide.

Fig. 9. Alignment of deduced sequences of 2,2-dialkylglycine decarboxylase (this work) and rat mitochondrial ornithine transcarbamylase (Maunakea and Pfeifer, 1983). A modification of the method of Needleman and Wunsch (1970) with a window of 50, a gap penalty of 10, and a site penalty of 2 was used. Identical residues or conservative substitutions are boxed. Active site residues are marked by dots.
Pseudomonas cepacia 2,2-dialkylglycine decarboxylase. Sequence and expression in Escherichia coli of structural and repressor genes.
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