Identification and Sequencing of a Gene Encoding a Hydantoin Racemase from the Native Plasmid of *Pseudomonas* sp. Strain NS671

KEN WATABE,* TAKAHIRO ISHIKAWA, YUKUO MUKOHARA, AND HIROAKI NAKAMURA
Odawara Research Center, Nippon Soda Co., Ltd., 345 Takada, Odawara, Kanagawa 250-02, Japan

Received 19 December 1991/Accepted 12 March 1992

DNA fragments containing the genes involved in the conversion of 5-substituted hydantoins to their corresponding L-amino acids have been cloned from the 172-kb native plasmid (pHN671) of *Pseudomonas* sp. strain NS671. The largest recombinant plasmid, designated pHPB14, encoded the ability to convert D-5-substituted hydantoins to the corresponding L-amino acids, whereas the smallest one, designated pHPB12, encoded the ability to convert them to their corresponding N-carbamyl-D-amino acids. Restriction analysis suggested that the inserts of both recombinant plasmids are derived from the identical portion in pHN671 and that the insert of pHPB14, compared with that of pHPB12, has an extra 5.3 kb in length. DNA sequencing revealed that pHPB14 contains two additional complete open reading frames, designated ORF5 and *hyuE*. Analysis of deletion derivatives of pHPB14 indicated that *hyuE* is required for the ability to produce L-amino acids from the corresponding D-5-substituted hydantoins, but ORF5 is not. Cells of *Escherichia coli* transformed with a plasmid containing *hyuE* were capable of racemizing different 5-substituted hydantoins, indicating that *hyuE* is a gene encoding a hydantoin racemase.

Racemic 5-substituted hydantoins are intermediates in the conventional chemical synthesis of corresponding racemic amino acids, but they can be converted to the corresponding L-amino acids by some bacteria belonging to the genera *Bacillus* (12, 15, 17), *Flavobacterium* (5, 8), and *Arthrobacter* (10), etc. The bacterial conversion is outlined in Fig. 1 (5, 10, 11, 15, 16, 19).

We have cloned DNA fragments containing the genes involved in the conversion of 5-substituted hydantoins to the corresponding L-amino acids from the 172-kb native plasmid (pHN671) of *Pseudomonas* sp. strain NS671 (13). Restriction analysis of the recombinant plasmids suggested that their inserts are derived from the identical portion in pHN671. The smallest recombinant plasmid, designated pHPB12, was investigated further, and three complete open reading frames (ORFs) (*hyuA*, *hyuB*, and *hyuC*) were found in its insert. Analysis of deletion derivatives of pHPB12 indicated that both *hyuA* and *hyuB* are involved in the conversions of D- and L-5-substituted hydantoins to corresponding N-carbamyl-D- and N-carbamyl-L-amino acids, respectively, and that *hyuC* is involved in the conversion of N-carbamyl-L-amino acids to L-amino acids (13). Thus, *Escherichia coli* carrying pHPB12 converts D-5-substituted hydantoins only to the corresponding N-carbamyl-D-amino acids. On the other hand, we found that *E. coli* carrying the largest recombinant plasmid, designated pHPB14, was able to convert D-5-substituted hydantoins to the corresponding L-amino acids. The insert of pHPB14 had an extra 5.3 kb in length, as compared with that of pHPB12. In this article, we describe the identification and sequencing of an additional gene required for this conversion. It was found that the gene is responsible for the racemization of 5-substituted hydantoins.

MATERIALS AND METHODS

Bacterial strains, plasmids, culture conditions, and chemicals. The vectors for cloning and sequencing were pUC18 and M13mp18, respectively (18). The DNA cloning was described previously (13). The largest recombinant plasmid (pHPB14) and the smallest one (pHPB12) were used. *E. coli* JM103 (4) was used as a host for recombinant plasmids. *E. coli* JM103 carrying a recombinant plasmid was grown in LB medium (3) supplemented with 50 µg of ampicillin per ml at 30°C. Optically active 5-substituted hydantoins and N-carbamyl-amino acids were prepared from the corresponding D- or L-amino acids (9) in our laboratory.

Conversions of 5-substituted hydantoins and N-carbamyl-amino acids with whole cells. *E. coli* JM103 carrying a recombinant plasmid was grown in 1.5 ml of LB medium with shaking at 30°C. Isopropyl-β-D-thiogalactopyranoside (IPTG; 7.5 µl of a 200 mM solution) and optically active 5-(2-methylthioethyl)hydantoin or N-carbamylmethionine (150 µl of a 2% solution) were added to the culture at an A_{600} of about 0.2 (1.3×10^8 cells per ml), and incubation was continued for 16 h for 5-(2-methylthioethyl)hydantoin or for 3 days for N-carbamylmethionine. The culture medium was analyzed by thin-layer chromatography on silica gel 60 F-254 (Merck) with a solvent system of 1-butanol–acetic acid–water (9:1:1, by volume), and the products were detected by the iodoplatinate method (14).

DNA sequencing. The 4.18-kb *Eco*RI fragment of pHPB14 (6.95-kb to 11.13-kb positions in Fig. 3) was subcloned in both orientations into the *Eco*RI site of M13mp18. Progressive unidirectional deletions of the insert DNAs were created by the method of Henikoff (1) by using a deletion kit (Takara Shuzo, Kyoto, Japan). These recombinants were transfected into *E. coli* JM103 to produce the single-stranded templates. Nucleotide sequences of the clones were determined by the dideoxy chain termination method of Sanger et al. (7) by using a Sequenase kit (U.S. Biochemical Corp., Cleveland, Ohio). Sequence analysis was totally carried out for both strands. For computer analysis, SDC-Genetyx

* Corresponding author.

FIG. 1. Bacterial conversion of racemic 5-substituted hydantoins to L-amino acids.

software (Software Development Co., Tokyo, Japan) was used.

Gene product identification. Cell extracts were prepared as described previously (13) from IPTG-induced *E. coli* JM103 carrying recombinant plasmids and analyzed with a 12% polyacrylamide gel by the method of Laemmli (2). After electrophoresis, the gel was stained with Coomassie brilliant blue. Protein standards used for molecular mass estimation were phosphorylase *b* (94.0 kDa), bovine serum albumin (67.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (30.0 kDa), and soybean trypsin inhibitor (20.1 kDa) (electrophoresis calibration kit; Pharmacia LKB, Uppsala, Sweden).

Racemization of 5-substituted hydantoins with whole cells. pKPN17 is a pUC18 derivative containing the *hyuE* gene of strain NS671 under the control of the *lac* promoter (see Fig. 4). *E. coli* JM103 carrying either pKPN17 or pUC18 was grown in 50 ml of LB medium with shaking at 30°C. IPTG (250 μ l of a 200 mM solution) was added to the culture at an A_{600} of about 0.2, and incubation was continued for another 2 h. The cells were harvested by centrifugation and suspended in 5 ml of 20 mM Tris-HCl (pH 7.5) containing 0.1 M NaCl. Ten A_{600} units of this suspension was centrifuged, and the precipitate was resuspended in 10 ml of 20 mM Tris-HCl (pH 7.5) containing 0.1 M NaCl and 0.2% of either D- or L-5-substituted hydantoins. After incubation with shaking at 30°C for various lengths of time, an aliquot of the suspension was centrifuged and the supernatant was filtered with a disposable filter (Acro LC3A; Gelman Sciences). The filtrate was analyzed by high-pressure liquid chromatography (HPLC) with a Chiralpak WH column (Daicel Chemical Industries, Tokyo, Japan) or by polarimetry with a digital polarimeter (DIP-360; Japan Spectroscopic Co., Tokyo, Japan).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to GenBank and assigned the accession number M84731.

RESULTS

Difference in conversion ability between pHPB14 and pHPB12. DNA fragments containing the genes involved in the conversion of 5-substituted hydantoins to their corresponding L-amino acids have been cloned from the 172-kb native plasmid (pHN671) of strain NS671 (13). The largest recombinant plasmid and the smallest one were designated pHPB14 and pHPB12, respectively. The conversion ability encoded by pHPB14 was compared with that encoded by pHPB12 (Fig. 2). Both L-5-(2-methylthioethyl)hydantoin and N-carbamyl-L-methionine were converted to methionine by

FIG. 2. Comparison of the conversion abilities encoded by pHPB14 and pHPB12. *E. coli* carrying either pHPB14 (a) or pHPB12 (b) was incubated with 5-(2-methylthioethyl)hydantoin (lanes 1 and 2) or N-carbamylmethionine (lanes 3 and 4), and the resultant culture media were analyzed by thin-layer chromatography. Lanes 1 and 3 show the D-isomer of each substrate, and lanes 2 and 4 show the L-isomer of each substrate. HYD, 5-(2-methylthioethyl)hydantoin; NCA, N-carbamylmethionine; MET, methionine.

E. coli carrying either pHPB14 or pHPB12. These methionines were found to be the L-isomer by thin-layer chromatography by using a Chiral plate (Macherey-Nagel). *E. coli* carrying pHPB14 converted D-5-(2-methylthioethyl)hydantoin to methionine (the L-isomer), whereas that carrying pHPB12 converted it to N-carbamylmethionine, which was found to be the D-isomer (judging from the configuration of the methionine produced chemically from it by the sodium nitrite method [16]). N-Carbamyl-D-methionine was not converted by either strain. These results indicate that *E. coli* carrying pHPB14 can racemize 5-(2-methylthioethyl)hydantoin and that the conversion of D-5-(2-methylthioethyl)hydantoin to N-carbamyl-D-methionine is blocked in this strain.

Analysis of deletion derivatives of pHPB14. pHPB14 contained an insert of 12.77 kb. Its restriction map is shown in Fig. 3. The restriction profile of the region from the pUC18 vector to the *Bam*HI site at the 7.47-kb position was identical with that of pHPB12 (13).

To determine the region which is required for the conversion of D-5-substituted hydantoins to the corresponding L-amino acids, we constructed some deletion derivatives of pHPB14 as follows. *Sal*I-digested pHPB14 was digested with *Afl*II, *Sna*BI, *Spl*I, and *Xho*I, independently, and the fragments were treated with Klenow fragment to produce blunt ends. These fragments were self-ligated. The desired recombinant plasmids were verified by restriction analysis and designated pAFL116, pSNA101, pSPL90, and pXHO84, respectively. pHPB14 was digested with *Sph*I, and the fragment was self-ligated to yield pSPH88. The deletion derivatives obtained as described above are summarized in Fig. 4.

The conversions of D-5-(2-methylthioethyl)hydantoin by *E. coli* carrying these deletion derivatives were examined (Fig. 5). The deletion derivatives having the insert to the *Sna*BI site or beyond maintained the ability to convert it to methionine (the L-isomer), while those lacking the insert to the *Spl*I site or beyond lost this ability. Therefore, we decided to determine the nucleotide sequence between the *Bam*HI site at the 7.47-kb position and the *Sna*BI site at the 10.09-kb position in the insert of pHPB14 (Fig. 3).

Nucleotide sequence analysis. The sequence from the *Bam*HI site at the 7.47-kb position to the *Sna*BI site at the 10.09-kb position in the insert of pHPB14 is shown in Fig. 6. Analysis of the sequence revealed three ORFs, designated

FIG. 3. Structure of pHPB14. The thick line represents the fragment from pHN671. The thin line represents the pUC18 vector. The numbers appended to the names of the restriction endonucleases indicate the distances from the start of the insert in kilobases. The regions of the complete ORFs found in the insert are indicated by arrows.

ORF5, ORF6, and ORF7. ORF5 was truncated, and this ORF was the 3' portion of the ORF5 found in the insert of pHPB12 (13). Complete ORF5 is predicted to encode a peptide of 456 amino acids, with a molecular mass of 50.6 kDa. ORF6 started with two adjacent ATG triplets (positions 1100 and 1106), and a possible ribosome-binding site (AGGA GGA) was present upstream of ORF6 (positions 1091 to 1097). When the second ATG triplet is used as an initiation codon, ORF6 is predicted to encode a peptide of 249 amino

FIG. 5. Conversions of D-5-(2-methylthioethyl)hydantoin by *E. coli* carrying the deletion derivatives of pHPB14. *E. coli* carrying the deletion derivatives of pHPB14 defined in Fig. 4 were incubated with D-5-(2-methylthioethyl)hydantoin, and the resultant culture media were analyzed by thin-layer chromatography. HYD, 5-(2-methylthioethyl)hydantoin; NCA, N-carbamylmethionine; MET, methionine.

acids, with a molecular mass of 27.1 kDa. ORF7 was truncated, and only the 3' portion of it (128 amino acids) was present in the sequence in Fig. 6. Downstream of ORF6, a palindromic sequence (positions 1918 to 1950) followed by a run of T residues, which is typical of the ρ -independent transcriptional termination signals (6), was found.

ORFs required for the ability to convert D-5-substituted hydantoins to corresponding L-amino acids. The nucleotide sequence analysis indicated that pSNA101 contains complete ORF6, whereas pSPL90, pSPH88, and pXHO84 are deficient in ORF6. Therefore, it was concluded that ORF6 is required for the ability to convert D-5-substituted hydantoins to the corresponding L-amino acids.

To determine whether ORF5 is also required for this ability, we eliminated ORF5 from pSNA101 in the following manner. pSNA101 was digested with *KpnI*, and the fragment was self-ligated to yield pKPN17 (Fig. 4), which contains complete ORF6. The 6.37-kb *EcoRI* fragment, containing *hyuA*, *hyuB*, and *hyuC*, was isolated from pHPB12 and

FIG. 4. Structures of the deletion derivatives of pHPB14. The closed boxes represent the pUC18 vector, the open boxes indicate insert DNAs, and the thin lines indicate deleted regions. Restriction endonucleases used to construct the deletion derivatives are shown at the top of the figure. The regions of the complete ORFs found in the insert are indicated by arrows.

ORF5→
 G S M L L F T I I G L T S G V A T G T W N P I D A M I Q T M G K D N 34
 GGATCCATGCTACTCTTTAGCATTCGGTTAACGAGTGGGGTTGCAACGGGAACATGGAATCCAATTGATGCTATGATCCAAACAATGGGAAAAGATA 100

P F L L I V C L A F V I L A Q W S T N I S A N L L P P G V I I V N 67
 ACCCTTCCCTTTAATGATGTTAGCCCTTGTATTTTAGCTCAATGGCTACAAATATTAGTCCGAATCTATTGCCCTCCAGGTTATATCATAGTAA 200

L F P R K I S F P M G A I I A G I I G L L I Q P W N Y A E F V P Q 100
 CCTTTTCCACGTAAGATATCATTTCCGATGGGTGCGATAATAGCAGGGATTATTGGATTACTTATTCAACCATGGAATTATGCGGAGTTGTCTCTCAA 300

I L M V I T A T L A P I V G I M F T D Y Y L L R K R R L N L D E L Y 134
 ATTCTAATGGTTATAAACAGCCACTAGCCCAATCGTAGGTATTATGTTTACCGATTATTATTGCTTAGAAAAACGAGGCTGAACCTTAGATGAACGT 400

K V D G Q Y K Y W K N V N P A A I I A Y I P S G L S V L L F P D Y 167
 ATAAAGTCGATGCTCAATATAAGTATTGGAATAATCCCGACCCATCATTCATACATCCATCCGTTTGTGAGTCTTTTGTTCCTGATTA 500

G F V T A L L I S M V L Y Y T L M K Y W I C K I Y E Q P E I T D A 200
 TGGATTGTGTACAGCATTTGTAATTTCTATGGTTTGTACTATACCTTAATGAAATATTGGATTGCAAGATCTATGAACAGCCTGAAATTACCGATGCA 600

N F N I K S Q G F T K E G Stop 213
 AATTTCATATAAAAGTCAAGGATTACGAAGAGGGATAATGTATAAAAAATTAAGTAATTGACCCGAGAAATGACGAATGGCATTGTACACAGGTCA 700

AAAAGTGAGCTGGATCGACATACAGATCATTACGGTACCCGAAATGGGTACGCATCAATTGAATCCTACTATGATGGAATACTTGAGTATTCTCGAG 800

TACTCCTGAAAAATACAAAGAAATTTAGAGGGCACTATACCTTCAGTTGAGGAAGGTAAAGTTACTAGGTTGTGCCGGTATGGCGGATTTAACCGAAAAAC 900

TCGAGAAAGAACTGGGGTACCTGAAATTTGATGGTGTAGTAGTGATTGATAGTTTATAGAAACAATTGTTGACCCCTTGGAAAAACAAGCAAACTAAAA 1000

CTTATAAATCTCCTGAAAGAAAGAAATATTGCGGAAATTAAGAAATTTGGGTCCGTGCAAAAAGCTATAAAATAAATTCATAAATCTAGGAGGAAAA 1100

ORF6(*hlyB*)→
 M K I K V I N P N T T L A M T K G I E H A A K S A A R S D T Q I 32
 TGGTTATGAAATTAAGTTATCAACCCCAATACAACCTTGGCAATGACAAAGGAATTGAACATGCTGCTAAGTCAGCTGCAAGATCTGATACCTAAAT 1200

V A V S P K M G P A S I E S Y Y D E Y L S I P G V I E E I K K G E 65
 TGTTCAGTGAGTCCTAAAATGGGTCCGGCTTCAATTGAATCCTACTATGATGAATATTTAAGCATTCCAGGAGTAATTGAGGAAATTAAGGAGGAGAA 1300

E E G V D A F V I A C W G D P G L H A A R E V T D K P V V G I A E S 99
 GAAGAAGGAGTAGATGCAATTTGTATAGCATGCTGGGGAGACCCGTGGATTGCATGCTGCGAGAGAAGTAACAGATAAACAGTTGTAGGCATTGCGGAAT 1400

S V Y L A S M L A A R F S V V T V L P R I K T M L E D L V D S Y G 132
 CCTCCGTTTATTAGCATCAATGCTTGACGCCAGATTTCCGTTGGTCACAGTTTACCTAGAATTAAACAATGTTAGAAGACCTGGTGTATCATACGG 1500

M Q K R V L N I R T T P M G V L D F E R D P E A G I E M L R Q E G 165
 TATGCAAAAACGTGTACTAAACATCCGTACGACACCGATGGGCCATTAGATTGTTGAGAGAGATCCAGAAGCGGGAATTGAAATGTTAAGGCAGGAAGGG 1600

K R A V E E D N A E A I L L G C A G M A E F A D S L E K E L G V P V 199
 AAAAGAGCGGTAGAGGAAGATAATGCAGAGCTATTTTACTTGGATGCTGCTGGTATGGCAGAATTGCGGATAGCCCTGAAAAAGAAATTAGGAGTTCGCC 1700

I D G V V A G V K F A E T I V D L G K K T S K L K T Y K Y P E K K 232
 TTATCGATGGAGTGTAGCGGGTGTGAAATTCGCCGAAACAATTGTTGATCTAGGAAAGAAAAACAAGTAACTAAAACTTATAAATATCCAGAGAAAA 1800

E Y V G A L E N F G R N Q T T T K Stop 249
 AGAATATGTTGGGCAATTGAGAACTTTGGACGAATCAACAACAACAAAAATTAAGAGTTTTCACACTGTTAAATCATAGATAGTTTATA 1900

TCAAATTTTATTAATTTTGGGCGAATTTCAATTACTAGTGAATTGCCCATTTTAAAGCATAACAGGGATTTCAGTGATATTGACTGTTAGGCAGAA 2000

CAGAAATTTTATATACATCCCTTGAATGAATTTATGCATAGCAATGGGTGAACCTATAAAAGTTCTTACATCAATATGTATCAAAAAGGAGTGCGATT 2100

TTCATGGATTTAAATCTAACAGGTCATCAATCCAGATCCAAATAGGATTCAACAACAATAATTTTAAATCATAAAAACAAGCTTTTCTAAAGAGGAAG 2200

CAGCAGCGATAGCTTTACTTTTAGGCATTGATTTAATAAATGCAAGTTTGATTAGATGAGTTTGGATGGGAGTAAATACCGAGCTTGAACATGGAAA 2300

Stop Y I C T Q N L H T K S P L L Y R A Q V H F 108

AGTATCCAGCCAAACAATGTAAGTGGTGATGATCCAAATGTTACAGAGATCCGATTAACTTCTAGCTACATTTGGATTATCATTAAATACCTTTATCAA 2400

L I W G F L H L Q H D L T V S I G I L R R A V N P N D N I V K D 75

TAATATCTTTTGTATCTTTACTACCAAGAGATTTGATATATTTTAAAAACAGTAAATGTTATCCAACTACAAAAATTAACAAAAATTTCTTAGCGAT 2500

I I D K T D K S G L S K S I N K L V T F T I W S C F N F L F K K A I 41

ATCTTCCACTACTTTTTCATCTTTTTCATCAAGATTTGAGATTAAAGTAATAAACTGTTCTTCTAGCATGTAGTCTTTATCAGTTAGTACTTGATCT 2600

D E V V K E D K E D L N Q S K L Y Y V T E E L N Y D K D T L V Q D 8

AGGAAAACGTTTAAATACGTA 2622
 L F V N L I R 1
 ←ORF7

FIG. 6. Nucleotide sequence of the 2.62-kb *Bam*HI-*Sna*BI region of the insert of pHPB14 and deduced amino acid sequences of ORFs. Nucleotides are numbered at the right of the sequence lines, with nucleotide 1 corresponding to the first nucleotide of the *Bam*HI site. Amino acids are numbered at the right of the sequence lines, with amino acid 1 corresponding to the first amino acid of each ORF.

FIG. 7. Identification of the ORF6 product. The cell extract from *E. coli* carrying either pKPN17 or pUC18 was analyzed by SDS-polyacrylamide gel electrophoresis. An additional peptide band found in the cells carrying pKPN17 is indicated by an arrow.

ligated into the *Eco*RI site of pKPN17. The orientation of the insert was examined by restriction analysis, and the recombinant plasmid with the insert in the same orientation to the *lac* promoter was designated pECO81 (Fig. 4). *E. coli* carrying pECO81 converted D-5-(2-methylthioethyl)hydantoin to methionine (the L-isomer) as shown in Fig. 5,

indicating that ORF5 is not required for the ability to convert D-5-substituted hydantoins to the corresponding L-amino acids.

Identification of the ORF6 product. The cell extract from *E. coli* carrying either pKPN17 or pUC18 was analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Fig. 7). An additional peptide band with an apparent molecular mass of 32.1 kDa was found in the cells carrying pKPN17. Thus, this 32.1-kDa peptide is thought to be the ORF6 product, although the value of the apparent molecular mass is somewhat higher than the value of the calculated molecular mass of the ORF6 product (27.1 kDa).

Function of the ORF6 product. *E. coli* carrying pKPN17 converted D-5-(2-methylthioethyl)hydantoin to neither *N*-carbamylmethionine nor methionine (Fig. 5). However, when D-5-(2-methylthioethyl)hydantoin was incubated with both *E. coli* carrying pKPN17 and that carrying pHPB12 at the same time, it was completely converted only to methionine (the L-isomer). On the other hand, when D-5-(2-methylthioethyl)hydantoin was incubated first with *E. coli* carrying pKPN17 and the supernatant was then incubated with that carrying pHPB12, it was converted to both *N*-carbamylmethionine and methionine. The molar ratio of these two products approached 1:1 with increasing incubation time with *E. coli* carrying pKPN17 (data not shown). These results indicate that the ORF6 product can function in a different cell from that containing the *hyuA*, *hyuB*, and *hyuC* products, and it is a hydantoin racemase. Thus, ORF6 was renamed *hyuE*.

Racemization of different 5-substituted hydantoins. IPTG-

FIG. 8. Racemization of different 5-substituted hydantoins by *E. coli* carrying pKPN17. *E. coli* carrying either pKPN17 (solid lines) or pUC18 (broken lines) was incubated with either the D-isomer (closed circles) or the L-isomer (open circles) of a 5-substituted hydantoin. The supernatant was analyzed by polarimetry at the points of time indicated. (A) 5-(2-Methylthioethyl)hydantoin; (B) 5-isopropylhydantoin; (C) 5-isobutylhydantoin; (D) 5-benzylhydantoin.

induced cells of *E. coli* carrying pKPN17 were incubated with different 5-substituted hydantoins. The supernatants were analyzed by HPLC with a Chiralpak WH column. The D- or the L-isomer of each substrate was racemized, and the molar ratio of the two isomers approached 1:1 with increasing incubation time. The total amount of the two isomers was constant during the incubation period, indicating that no degradation of 5-substituted hydantoins occurs under these conditions (data not shown).

Thus, we monitored the racemization by polarimetry, which is a simple and accurate method. The results are shown in Fig. 8. *E. coli* carrying pKPN17 can racemize 5-substituted hydantoins having either aromatic or aliphatic substituents.

DISCUSSION

We have cloned the DNA fragments containing the genes involved in the conversion of 5-substituted hydantoins to their corresponding L-amino acids from the 172-kb native plasmid (pHN671) of *Pseudomonas* sp. strain NS671. The largest recombinant plasmid (pHPB14) contained five complete ORFs, designated *hyuA*, *hyuB*, *hyuC*, ORF5, and *hyuE*. Both *hyuA* and *hyuB* are the genes for the DL-hydantoinase, and *hyuC* is the gene for the N-carbamyl-L-amino acid amidohydrolase (13). In this article, we have identified *hyuE* as the gene for the hydantoin racemase, while no function of the ORF5 product has been found.

E. coli carrying a plasmid which contained *hyuA*, *hyuB*, *hyuC*, and *hyuE* was able to convert D-5-(2-methylthioethyl) hydantoin only to L-methionine. Thus, the question arises why N-carbamyl-D-methionine is not produced by this strain. We believe that the reason should be derived from the nature of the DL-hydantoinase because of the following facts. When using racemic 5-(2-methylthioethyl)hydantoin as a substrate, *E. coli* carrying pHPB12, which contained *hyuA*, *hyuB*, and *hyuC*, converted the L-isomer first. The conversion of the D-isomer then took place when the conversion of the L-isomer was completed (unpublished data). Therefore, it is thought that D-5-(2-methylthioethyl)hydantoin is converted only to L-methionine in the presence of the hydantoin racemase. Further studies are necessary to examine the catalytic mechanism of the DL-hydantoinase by using a purified enzyme.

The deduced amino acid sequence of *hyuE* had no significant similarity to the known sequences in the NBRF (National Biomedical Research Foundation; release 26) and SWISS (European Molecular Biology Laboratory; release 15) protein data bases. The cloning and expression of *hyuE* in *E. coli* enabled us to purify the gene product hydantoin racemase. Work is now under way to characterize this enzyme.

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