Nucleotide Sequence of SHV-2 β-Lactamase Gene

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The nucleotide sequence of plasmid-mediated β-lactamase SHV-2 from Salmonella typhimurium (SHV-2pHT1) was determined. The gene was very similar to chromosomally encoded β-lactamase LEN-1 of Klebsiella pneumoniae. Compared with the sequence of the Escherichia coli SHV-2 enzyme (SHV-2E_cdo) obtained by protein sequencing, the deduced amino acid sequence of SHV-2pHT1 differed by three amino acid substitutions.

Transferable resistance to broad-spectrum cephalosporins, such as cefotaxime, is a developing phenomenon among members of the family Enterobacteriaceae and involves new β-lactamases which belong to class A in the scheme of Ambler (1) and are genetically derived from TEM or SHV enzymes (see reference 11 for a review). Since the discovery in 1983 of a plasmid-mediated β-lactamase related to SHV-1 (10), new SHV-type enzymes have been found, mainly in Klebsiella isolates (5, 7–9). Although the amino acid sequences of SHV-1 from Klebsiella spp. and SHV-2 from Escherichia coli A2302 (SHV-2E_cdo) have been determined (3, 4), little is known concerning the nucleotide sequence, the signal peptide, or the promoter of SHV-type enzymes. For this reason, we chose to sequence the bla gene coding for SHV-2 (blaSHV-2pHT1), which was isolated from Salmonella typhimurium 122 (14). In a previous study, we showed that this gene was carried by a 12.5-kilobase plasmid designated pHT1 and encoded an enzyme (SHV-2pHT1) which was indistinguishable from SHV-2E_cdo β-lactamase by isoelectric point, substrate profile, and kinetic constants (6). The blaSHV-2pHT1 gene was mapped to adjacent PstI fragments of 0.86 and 0.79 kilobases and cloned into plasmid pBR322. For DNA sequencing, the PstI fragments were subcloned into bacteriophages M13mp18 and M13mp19. In addition, deleted PstI fragments were prepared by Bal 31 exonuclease digestion (Boehringer Mannheim, Meylan, France) to produce inserts with overlapping sequences. The deleted fragments were further subcloned into the same vectors. The DNA sequence was determined on both strands by the dyeoxy-chain termination method described by Sanger et al. (12). We used the Sequenase sequencing kit from the United States Biochemical Corp., Cleveland, Ohio, as indicated by the supplier, and [α-32P]dATP (600 Ci/mM) was purchased from Amersham France, Les Ulis, France. Labeled DNA was analyzed by electrophoresis at 50 W in buffer gradient gels (8 M urea, 6% polyacrylamide).

Figure 1 shows the nucleotide sequence of the blaSHV-2pHT1 gene. An ATG codon at position 223 initiates a long open reading frame of 858 nucleotides which ends at position 1081 with a TAA codon. The initiation codon is preceded by a Shine-Dalgaro ribosome-binding sequence, AAGG, and a possible −10 region, TATTCT, and a −35 region, TTGCGA, of a promoter. The deduced sequence of 286 amino acids is shown in Fig. 2. The mature enzyme is 265 amino acids long and begins with a signal peptide of 21 residues. The sequence of SHV-2pHT1 shows a great similarity to that of chromosomally encoded β-lactamase LEN-1 of Klebsiella pneumoniae (2). These two proteins show 89% similarity in nucleotide sequence, and 234 amino acids are identical in the mature enzymes. LEN-1 β-lactamase is seven residues shorter than SHV-2pHT1 at the carboxy-terminal extremity. This is due to the deletion of a G residue at position 1054, which changes the reading frame and leads to a termination codon 7 nucleotides downstream in the LEN-1 sequence. If this deletion was disregarded, the deduced sequences of the last nine amino acids would be identical in the two enzymes. The similarity between LEN-1 and SHV-2pHT1 also includes the signal peptide in which 17 of 21 residues occupy identical positions. In addition to nucleotide similarity within the blasSHV-2pHT1 and LEN-1 genes, the sequence is highly conserved in the noncoding region which stretches to 73 base pairs upstream from the initiation codon and includes the possible −10 locus of a promoter. In contrast, similarity is lacking between the LEN-1 and SHV-2pHT1 nucleotide sequences surrounding the −35 promoter region. This could indicate that, although very closely related, these two enzymes do not share the same promoter.

Comparison of the amino acid sequence of SHV-2pHT1 β-lactamase from S. typhimurium with those of SHV-1 (4) and SHV-2E_cdo (3) shows four and three amino acid substitutions, respectively. Compared to SHV-1, SHV-2pHT1 and SHV-2E_cdo share the same substitution of serine for glycine at position 234. It has been reported that this mutation is the only change observed within the amino acid sequences of SHV-1 and SHV-2E_cdo and is responsible for the cepotaxime-hydrolyzing activity of SHV-2 β-lactamase (3). In contrast, SHV-2pHT1 differs from SHV-1 and SHV-2E_cdo by three additional substitutions: a glutamine (CAA) instead of a leucine (CTN, TTA, or TTG) at position 31, an alanine (GCC) instead of a threonine (ACN) at 136, and a threonine (ACC) instead of an alanine (GCN) at 137. Remarkably, these positions are occupied by the same three residues and are encoded by the same codons within the LEN-1 sequence. From the most likely codon that would be present for the SHV-2E_cdo enzyme, a single base change is sufficient to account for each substitution. Since SHV-2pHT1 and SHV-2E_cdo are indistinguishable by their enzymatic activities, these mutations are not likely to be involved in the catalytic activity, but they could cast light on the evolutionary relationships among the SHV-type enzymes. If all these enzymes are related to the progenitor LEN-1 β-lactamase, they could have evolved independently in different bacterial hosts. On the basis of sequence analysis, LEN-1 appears to

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**FIG. 1.** Nucleotide sequence of *bla*SHV-2(*pHT1*) gene. Nucleotides are aligned with the sequence of the LEN-1 enzyme (2); a colon represents identity. The initiation (*) and the stop codons are indicated. **** represents the beginning of the mature enzyme. Upstream from the *bla*SHV-2(*pHT1*) gene, SD represents a putative Shine-Dalgarno consensus sequence, and the possible −10 and −35 regions are underlined. The *PstI* site within the gene is indicated.
be more closely related to SHV-2<sub>pPE</sub> than to SHV-1 and SHV-2<sub>E.coli</sub>, in which three additional mutations have occurred. Lastly, the determination of the SHV-2<sub>pPE</sub> nucleotide sequence will allow the development of specific oligonucleotide probes and the performance of site-directed mutagenesis to study the effect of point mutations on enzyme activity.

We thank Michel Barthélémy and Jean Péduzzi for helpful discussion and preliminary communication of their data.

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