Nucleotide Sequence and Characterization of ptsG Gene Encoding Glucose-specific Enzyme II of Phosphotransferase System from Brevibacterium flavum

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Nucleotide sequence of Brevibacterium flavum ptsG gene capable of complementing Escherichia coli ZSC113 mutations defective to glucose permease activity of phosphotransferase system was completely determined, and the gene product was compared with other glucose-specific enzyme II (EIIS). A ptsG gene of B. flavum consisted of open reading frame of 2,025 nucleotides putatively encoding polypeptide of 675 amino acid residues and TAA stop codon. Deduced amino acid sequence of B. flavum EIIS had high homology with EIIS of Corynebacterium glutamicum, C. efficiens, and B. lactofermentum. Arrangement of structural domains, IBCA, of B. flavum EIIS protein was identical to that of EIIS belonging to glucose-phosphotransferase system.

Key words: Brevibacterium flavum, phosphotransferase system, glucose permease gene, nucleotide sequence, comparison

In bacteria, the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) is responsible for the uptake and phosphorylation of a number of carbohydrates. The cytoplasmic phosphoproteins, enzyme I (EI) and histidine-containing phosphocarrier protein (HPr), sequentially transfer a phosphoryl group from a phosphoenolpyruvate (PEP) to a membrane-bound EI, which catalyzes the concomitant transport and phosphorylation of the carbohydrates. The EIIS contain three or four structural domains IIA, IIB, and IIC and are divided into four classes according to their amino acid sequence similarities. Among them, EI proteins specific to glucose, sucrose or β-glucoside, and belonging to the glucose-PTS class, contain three domains IIA, IIB, and IIC. The domain IIA, which interacts with a phosphorylated-HPr, exists as a separate protein or as another domain-linked protein. The IIC domain comprises between six and eight putative transmembrane segments showing high hydrophobicity.

The Gram-positive corynebacteria, including Brevibacterium lactofermentum, B. flavum, Corynebacterium glutamicum, C. efficiens, and B. ammoniagenes, have been used for industrial production of various amino acids and nucleotides. The presence of two PTS systems specific for glucose or fructose was reported in B. flavum and C. glutamicum, from which the genes encoding glucose-specific EI (EIIS) were recently characterized. The EIIS of B. ammoniagenes, B. lactofermentum, and C. glutamicum contained IIA, IIB, and IIC domains in a single polypeptide with an arrangement of structural domains, IBCA, belonging to the sucrose/β-glucoside subgroup of the glucose-PTS class on the basis of their amino acid sequence alignments. The ptsG gene encoding EIIS was previously cloned from B. flavum into Escherichia coli. This work describes the sequencing of a complete B. flavum ptsG gene that codes for EIIS, and compares its deduced amino acid sequence with those of other bacterial strains.

Materials and Methods

Bacterial strains, plasmids and media. E. coli XL-1 blue (supE44 thi-1 relA1 gyrA96 proAB lacY1 F' [proAB lacZ M15 Tn10 (tetR)] ) was used as a host for subcloning experiments, and plasmid pUC19 was used for all cloning and sequencing experiments. MacConkey agar was used to select the E. coli transformant carrying recombinant plasmid. E. coli was cultured at 37°C in LB broth (10 g tryptone, 5 g yeast extract, 10 g NaCl per liter, pH 7.0). Ampicillin (50 g/ml) was used for the selection of transformants of E. coli.

DNA sequencing and computer analysis. The standard procedures of Sambrook et al. were used for DNA manipulation. Restriction endonucleases, protease, and RNase obtained from Boehringer Mannheim (Manheim, Germany), and T4 DNA ligase from Solgent Co. (Deajeon, Korea), were used as recommended by the manufacturers. Restriction endonuclease-generated DNA fragments of the cloned B. flavum chromosomal DNA were subcloned into pUC19. The nucleotide sequences of the fragments were determined with a DNA sequencer (ABI Prism 377, Perkin Elmer Co., Foster, CA, USA). The DNA and protein sequences were analyzed using the DNASIS (Hitachi Software Engineering, Tokyo, Japan) program.
Results and Discussion

Nucleotide sequence of the B. flavum ptsG gene. The ptsG gene of B. flavum had been previously cloned into pUC19 to form a recombinant plasmid pBF93 by in vivo complementation of an E. coli mutant strain lacking EIIGlc. For sequencing the B. flavum ptsG gene of pBF93, various restricted fragments were subcloned into pUC19. The 2.9-kb B. flavum DNA fragment of pBF93 was completely sequenced. The 2.025-nucleotide ptsG, beginning at position 228 by an ATG codon and terminating at position 2,255 by the ochre stop codon TAA, was identified in the nucleotide sequence (Fig. 1). The ptsG encodes a polypeptide with 675 residues and a calculated M_r of 71,982. The codon usage of ptsG exhibited an overall GC content of 52.1%, and a wobble-position GC content of 55.5%. The base composition of a B. flavum ptsG gene was similar to those of C. glutamicum (total GC content, 52.3%; wobble-position GC content, 54.6%) and B. lactofermentum ptsG (total GC content, 52.7%; wobble-position GC content, 55.5%). The GC contents of 34 genes from B. lactofermentum and C. glutamicum ranged from 50 to 62%. The ptsG gene of C. efficiens had a total GC content of 63.9%, with 83.1% GC content at the third base of the codon. The GAAAGG sequence element, six bases upstream from the first ATG start codon at nucleotide position 216-221, could be the ribosome-binding site for the mRNA. There is a 14-bp palindrome beginning 37 nucleotides downstream of the TAA stop codon. This element could be involved in the rho-independent termination of the ptsG gene transcription.

Based on the hydrophathy of the deduced amino acid sequence calculated using the method of Kyte and Doolittle (data not shown). EIIIGlc was predicted to contain a hydrophobic region (IIC domain) corresponding to amino acid residues from 120 to 465 and consisting of six sub-regions with an average hydrophathy exceeding 1.0, each comprised of 20 amino acid residues capable of spanning the cytoplasmic membrane. The amino-terminal part (6-84 aa) of the protein was relatively hydrophilic (IIB domain), and the carboxy-terminal part (526-649 aa) was relatively hydrophobic (IIA domain), indicating that the EIIIGlc of B. flavum has an arrangement of BCCA domains identical to those of EIIIGlc from B. lactofermentum, C. glutamicum, and B. ammoniagenes.

Fig. 1. Nucleotide sequence of 2,900-nucleotide B. flavum DNA fragment and deduced amino acid sequence. The undefined sequence preceding the ATG start codon of ptsG gene is a putative ribosome-binding site. The TAA stop codon is indicated with asterisks below the sequences. The inverted repeat is underlined by horizontal arrows. Numbers at the end of each line correspond to the nucleotide position. The sequence data have been submitted to the GenBank nucleotide sequence database under accession number DQ267153.
Comparison of the *B. flavum* EII<sub>Gl</sub>c sequence with other EIIs. Comparison of the deduced amino acid sequence of the *B. flavum* EII<sub>Gl</sub>c with those of other EIIs in the NCBI database using the BLAST search program<sup>15</sup> revealed the present EII<sub>Gl</sub>c was homologous to those belonging to glucose-PTS. The phylogenetic relationships based on amino acid sequences of *B. flavum* EII<sub>Gl</sub>c and other EIIs of glucose-PTS were investigated using CLUSTAL W software. The *B. flavum* EII<sub>Gl</sub>c exhibited high homologies with *C. glutamicum* EII<sub>Gl</sub>c (97%), *B. lactofermentum* EII<sub>Gl</sub>c (88.3%), and *C. efficiens* EII<sub>Gl</sub>c (81.6%). It was also homologous with the EIIs of *Propionibacterium acnes* (47.4% similarity), *B. ammoniagenes* (44.5% similarity), *C. diphtheriae* (44.3% similarity), and *Bifidobacterium longum* (38.1% similarity). The EII<sup>Gl</sup>c was also similar to the β-glucoside-specific EIIs of *Bacillus halodurans* (32.2% similarity), *B. licheniformis* (32.6% similarity), *Lactobacillus acidophilus* (31.7% similarity), and *L. plantarum* (31.3% similarity). Amino acid sequence of the *B. flavum* EII<sub>Gl</sub>c was aligned with those of three EIIs<sub>Gl</sub>c showing high homologies. Amino acid sequences of the linker region connecting IIA domain and IIB domain exhibited the lowest similarities among the EIIs<sub>Gl</sub>c (Fig. 3). The linker region of *B. flavum* EII<sub>Gl</sub>c consisted of Gly-Ala repeats (495–509), while IIA domain of the other EII<sub>Gl</sub>c was connected to the IIB domain by a PA linker consisting of Pro-Ala repeats. One histidine residue at position 594, homologous to the active site, His, of *E. coli* EII<sub>Gl</sub>c, which was phosphorylated by the phospho-HPr protein.<sup>16</sup> The residue was assumed to be a cystidyl residue (28 aa) in the IIB domain of the protein. The

Fig. 2. Phylogenetic analysis of 16S rRNA. Neighbor-joining tree showing the phylogenetic relationships based on amino acid sequences of glucose-specific EIIs and β-glucoside-specific EIIs of several bacteria including *C. glutamicum* (CAF21369), *C. efficiens* (NP_738068), *B. diphtheriae* (NP_939508), *B. lactofermentum* (AAA22992), *B. ammoniagenes* (AAC27701), *Propionibacterium acnes* (YP_055815), *Bifidobacterium longum* (ZP_00121506), *Bacillus halodurans* (BAH04015), *B. licheniformis* (AAU25634), *Lactobacillus acidophilus* (YP_193627), *L. plantarum* (NP_787373), and *B. flavum*. GenBank accession numbers are described in parenthesis. Below the tree scale indicates the number of nucleotide substitutions for protein sequences.

The *B. flavum* EII<sub>Gl</sub>c contained two amino acid residues, which mediate phosphate transfer from phospho-HPr to glucose. The sequence was compared with other EIIs<sub>Gl</sub>c and β-glucoside-specific EIIs.<sup>15</sup> One was a histidine residue at position 594, homologous to the active site, His, of *E. coli* EII<sub>Gl</sub>c, which was phosphorylated by the phospho-HPr protein. The other was assumed to be a cystidyl residue (28 aa) in the IIB domain of the protein.
EIIF of E. coli was phosphorylated at a Cys residue (421 aa) by a soluble phospho-EIIA. The amino acid sequence conservation (HCA TRLR) around the Cys residue of B. flavum EIIF was also shared by other EIIs specific for glucose, sucrose, and β-glucoside of glucose-PTS.

References