Short Communication

Analysis of the Escherichia coli gntT and gntU Genes and Comparison of the Products with Their Homologues

Mamoru Yamada, Takuya Kawai, and Hanae Izu

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan

Received May 13, 1996

The Escherichia coli gluconate permease genes, gntT and gntU, were cloned and characterized. At least four homologues to GntT were found in E. coli by database searching. These proteins including GntT and GntU appear to have similar topological structures with 14 membrane-spanning segments, suggesting that they constitute a GntP family.

Key words: gluconate permease; GntP family; GntU: GntT

In the gluconate uptake and catabolism of Escherichia coli, the GntT system is known to function predominantly at the initial step, which appears to be supported by the GntII system as a subsidiary. Three genes, gntT, gntU, and gntS, encoding gluconate permeases in the two systems have so far been identified genetically and mapped on the genome. Recently, the gntP gene encoding another gluconate permease was also shown to be involved in gluconate uptake in E. coli.

To discover the physiological function of those permeases in gluconate metabolism, we subcloned the gntT gene and analyzed the gntT and gntU genes using conventional recombinant DNA techniques. The gntT gene was reported to occur between the bioH and malA loci and near the gntRKU genes. Thus, from a Kohara phage clone, E3C10, a mini-library was made and introduced into a strain, YU120 (H. Izu et al., submitted), lacking gntT. A clone with the positive fermentation was then isolated on EMB plates containing 0.5% gluconate. From the clone, a 2.0-kb fragment was subcloned into pBR322. The resulting clone, pGNNT20, showed a positive gluconate fermentation in YU120 and also in a strain, Hfr G6MD2 [hisA323, tria[aac(3)ID]] provided by P. Postma, which deletes the region including gntT. The nucleotides of the inserted DNA were then sequenced, and one ORF was found in it, suggesting that GntT consists of 437 amino acid residues with a molecular mass of 45.9 kDa. The sequence obtained here was found to match completely with that of orf-0437 in the GenBank database, which was analyzed by the genomic sequencing project. GntT has significant sequence similarity (50%) to the Bacillus subtilis gluconate permease (GntP) as shown in Fig. and Table.

We have cloned the gntRKU operon, and characterized its gene organization and expression. (H. Izu et al., submitted). Here, all of the nucleotides of gntU were sequenced. The initiation codon for GntU with a possible ribosome recognition sequence was found after gntK, and the ORF with 448 codons is followed by a possible ρ-independent terminator. The nucleotide sequence of the region including gntU appeared in the databases during the time we sequenced its nucleotides. From sequence comparison, it was found that one base (CGAAGCGTT) at the 332th amino acid residue of GntU in our sequence was absent in the sequence of the databases. Based on our sequence, GntU was deduced to consist of 447 amino acid residues with a molecular mass of 46.4 kDa. The protein shows 36% and 35% similarity to the B. subtilis GntP and to GntT, respectively. The lack of one base may result from a sequencing error because the protein deduced from the databases had a much shorter C-terminus than GntT, and lower similarity.

Searching for homologues in E. coli to GntT was done using the SWISS-PROT database, and four proteins, DsDC, YjiB, YigT, and Yjhf, in addition to GntU were uncovered (Fig.). Out of the proteins, DsDC and YjiB were reported to be a β-serine permease in the database and a transporter (encoded by the gntP gene) with high affinity for gluconate, respectively, and the other two were found to be uncharacterized previously. DsDC, YjiB, and YigT have 35%, 40%, and 61% similarity to GntT, respectively, and 36%, 37%, and 50% to the B. subtilis GntP, respectively (Table). The remaining one, Yjhf, was also significantly similar to GntT, but its reported size was about 50 amino acid residues shorter than that of GntT. We thus carefully searched three different frames on the reported nucleotide sequences, finding one possible frame shift at the C-terminus, which seems to be caused by lacking one base at the site corresponding to the 354th amino acid residue of the revised Yjhf. Because the C-terminal sequence of the revised protein was significantly similar to those of the homologues including GntT, we assumed that the lacking of one base was due to failing in nucleotide sequencing. The revised Yjhf has 48% and 60% similarity to GntT and the B. subtilis GntP, respectively. In Haemophilus influenzae Rd, a homologue, Gntph, to GntT was also found by database searching but has a slightly longer C-terminus than the others (Fig.). On the basis of the similarity to the

<table>
<thead>
<tr>
<th>Protein</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GntT</td>
<td>61</td>
<td>50</td>
<td>48</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>YigT</td>
<td>50</td>
<td>48</td>
<td>40</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>GntPh</td>
<td>60</td>
<td>37</td>
<td>36</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Yjhf</td>
<td>36</td>
<td>33</td>
<td>33</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>YjiB</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>GntU</td>
<td>35</td>
<td>30</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DsDC</td>
<td>35</td>
<td>30</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Proteins are as described in the legend to Fig.

* To whom correspondence should be addressed. Telephone: 81 839 33 5869, Fax: 81 839 33 5820, E-mail: yamada@agr.yamaguchi-u.ac.jp
Fig. Sequence Alignment of Glucan Permeases, GntT and GntU, with Their Homologues.

Sequence alignment was done by using the MAFFT program of GENETYX (Software Development, Tokyo, Japan). GntT, GntP, and GntU are a high-affinity glucan permease in E. coli, respectively. YigT, YipB, and DscD were selected as homologues in E. coli to GntT from the databases and GntPb also selected as a homologue in H. influenzae Rd from the databases. rYJH was a sequence revised from YjHF in the databases. DscD and YipB were reported to be o-serine permease in the databases and a high-affinity glucan permease, respectively, and YigT and YjHF were uncharacterized previously. Asterisks show amino acid residues conserved among the proteins.

B. subtilis GntP, it is suggested that those proteins, including GntT and GntU, constitute a GntP family.

To examine whether those proteins have topological structures similar to that of the B. subtilis GntP or not, their hydrophathy plots were compared (data not shown). They show the profiles resembled to each other and have 14 hydrophobic segments, enough to span the E. coli cytoplasmic membrane. Therefore, it is suggested that they form similar structures in the membrane, and that YigT and YjHF may also function as transporters. It is noteworthy that the GntP family may be unique in respect of having 14 membrane-spanning segments since a large family of transporters have 12 membrane-spanning segments. Therefore, the family may be classified into one
subgroup of the entire transporters.

The functions and gene organization of those homologues were deduced according to the EMBL database. The \textit{yfgT} gene is located close to the \textit{yfgS}, \textit{yfgU}, \textit{yfgV}, and \textit{gntV} genes at the \textit{pepa-gntV} loci at 96.7 min. GntV and YfgS show 47% similarity to a gluconate kinase, GntK, in the GntI system and 46% similarity to a repressor, GntR, for genes in the system, respectively. Moreover, YfgU shows 42% similarity to 2-deoxy-D-gluconate 3-dehydrogenase, KduD, in \textit{Erwinia chrysanthemi}. Therefore, they might be constituents of the GntII system, and especially, it is possible that GntV and YfgS are a gluconate kinase and a repressor for the system, respectively. The \textit{yfgT} gene might be the \textit{gntS} gene encoding a high affinity gluconate permease in the GntII system because it is located near \textit{gntV} and \textit{yfgS} and no ORF similar to GntT was found between \textit{aidB} and \textit{rpsF} near 95.3 min where \textit{gntS} was originally mapped. On the other hand, the \textit{yfhF} gene occurs at 97 min, where no such gene related to sugar metabolism has been reported. Since YfhF is located the most closely to the \textit{B. subtilis} GntP in the phylogenetic tree (data not shown), it may be another gluconate permease in \textit{E. coli}. For identifying the physiological functions and roles of the genes discovered here, biochemical and molecular genetic experiments are required, which are necessary for understanding the gluconate uptake and catabolism of \textit{E. coli}.

**References**