The glucitol permease of *Escherichia coli*: a tripartite permease of the phosphotransferase system

In 1987, a report from this laboratory described the primary structure of the glucitol permease of *Escherichia coli* (5). This permease (GenBank accession no. J02708; SWISS-PROT identifier P05705) appeared to be unique in its domain structure since in contrast to all of the phosphoryl transfer permeases of the phosphoenolpyruvate:sugar phosphotransferase system (PTS), it appeared to have its IIB domain sandwiched in between the two halves of the IIC domain in a single polypeptide chain (3). The gene encoding this protein was designated *gutA* (5). The IIA domain was a distinct polypeptide chain encoded by a distinct gene, *gutB*.

Recently, two glucitol PTS permeases have been sequenced, one from the close *E. coli* relative *Erwinia amylovora* (accession no. Y14603; 1) and the other from the Gram-positive bacterium *Clostridium beijerinckii* (accession number AJ002527; 4). Both of these permeases were found to consist of three, rather than two polypeptide chains. Aldridge et al. (4) referred to the two genes encoding the equivalent of the putative GutA protein of *E. coli* as the *srlA* and *srlB* genes, whereas the gene encoding the IIA domain was referred to as *srlB*.

Recently, Blattner et al. (2) and Yamamoto et al. (7) reported the sequence of the *E. coli* glucitol operon as part of *E. coli* genome sequencing projects (accession numbers AE000354 and D90892, respectively). Their results reveal the probable presence of an erroneous frameshift mutation reported by Yamada & Saier (5) at position 1074. An extra G appeared to have been inserted at this position. Reinspection of the original sequencing gel used by Yamada & Saier (5) indeed revealed a faint band at position 1074 corresponding to G. However, no corresponding C on the opposite strand was observed, and the weak band corresponding to G at position 1074 did not appear in a second, duplicate gel. These observations allow us to conclude that the correct sequence is that reported by Blattner et al. (2) and Yamamoto et al. (7).

The correct sequence of the *E. coli* glucitol permease reveals that the *E. coli* glucitol permease has the same tripartite polypeptide structure as those from *E. amylovora* and *C. beijerinckii*. Thus, *gutA* encodes a hydrophobic protein (187 residues) with four putative transmembrane α-helical spans (TMSs); *gutE* encodes a larger protein (319 residues) that includes the hydrophilic IIB domain fused to a hydrophobic putative 4 TMS domain, and *gutB* encodes the hydrophilic IIA domain (3). We note that no other sugar-specific PTS permease is known to possess an IIC domain which is encoded by two distinct genes.

An average hydropathy plot and an average amphipathic plot (with the angle set at 100° for α-helix) of the *gutA* and *gutE* gene products reveals the presence of striking peaks of amphipathy preceding both hydrophobic domains (T. Le, T.-T. Tseng & M.H. Saier, Jr, unpublished). As previous results have clearly suggested that the corresponding amphipathic helical structure in the mannotol Enzyme II of *E. coli* is required for proper insertion of the protein in the membrane (6), we suggest that the *gutA* and *gutE* gene products are independently inserted into the membrane using the same insertional machinery, dependent on an amphipathic α-helical structure preceding the first hydrophobic TMS. The revised sequences have been submitted to the GenBank/EMBL and SWISS-PROT databases.

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