Functional genomic studies of dihydroxyacetone utilization in Escherichia coli

Dihydroxyacetone (DHA), a triose intermediate in glycerol metabolism, can be utilized by Escherichia coli as a sole exogenous source of carbon and energy. In an early report, several lines of evidence suggested that a DHA-induced phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) was responsible for the uptake and phosphorylation of DHA in E. coli (4). First, using a spectrophotometric assay, PEP was converted to pyruvate in the presence of DHA. Second, ATP-dependent phosphorylation activity was defective for growth on and phosphorylation of DHA as sole carbon source and a single operon, the dha operon, was identified (4). Finally, a divergently transcribed DhaH gene b1201 (ycgU; here designated dhaR) was identified by PCR amplification, cloning and sequencing of the corresponding straight-chain carbon compounds such as DHA (3, 5). A glpF gene is also present in the genome of E. coli that might encode a DHA transport system. We presume that DHA can enter the cell via the glycerol facilitator, GlpF, a non-specific channel protein capable of transporting straight-chain carbon compounds. The similarity of both DhaK1 and DhaK2 to chemically established DHA kinases. The site of the transposon insertion in this mutant was mapped to the 26 min region of the E. coli chromosome between the dddA and hemA genes.

Fig. 1. Genes comprising the DHA utilization operon, dhaK1K2H, and its putative divergently transcribed regulatory gene, dhaR. The spacing between genes is presented in parentheses (nt). Currently accepted gene designations (ycg designations) and those proposed here (dha designations) are presented. The domain structure of the DhaH protein, showing a putative regulatory domain (R), an HPr-like domain (H) and the N-terminal portion of an Enzyme I-like domain (IΔ) of the PTS is shown below the dhaH gene.

DhaH are encoded in the incompletely sequenced genomes of Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus pneumoniae, Bacillus stearothermophilus, Corynebacterium diptheriae and Desulfovibrio vulgaris. All of these organisms encode DHA kinase homologues.

Additionally, homologues of the R domain of the DhaK1 and DhaK2 proteins similar to the N- and C-terminal halves, respectively, of the DHA kinases from Citrobacter freundii, Saccharomyces cerevisiae and other organisms. We have designated these genes dhaK1 and dhaK2, respectively. Immediately downstream from these genes, b1198 (ycgC; here designated dhaH), encodes a multidomain protein (DhaH) consisting of (i) an N-terminal segment of 144 residues (termed ‘R’), (ii) a central 88 residue segment (H) homologous to HPr of the PTS and (iii) a C-terminal 200 residue segment (IΔ) homologous to the N-terminal region of Enzyme I of the PTS that bear the HPr-recognition and histidyl phosphorylation sites of Enzyme I (7). DhaH therefore has the domain order R-H-IΔ (see Fig. 1). R is homologous to a small 133 residue protein, DRB0052, from Demococcus radio-durans (9) encoded adjacent to the two genes (dhaK1 and dhaK2) that together probably encode DHA kinase in this organism. Additionally, homologues of the R domain of the transposon insertion in this mutant was mapped to the 26 min region of the E. coli chromosome between the dddA and hemA genes.

REFERENCES

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variety of sources suggested the possibility that a sequencing error had introduced an apparent frameshift in a single gene. To investigate this possibility, we PCR-amplified, cloned and sequenced on both strands this region from three distinct E. coli K12 strains (MG1655, Hfr7 and DH5α). In all cases we found the sequences to be identical to that reported by Blattner et al. (1).

The region containing dhaK1 and dhaK2 was PCR-amplified and cloned into the His-tag vector pQE30 (Qiagen), thereby introducing a His tag onto the N-terminal end of DhaK1. Purification of the resulting fusion protein over a nickel column yielded a product of 40 kDa, as estimated by SDS-PAGE. This product corresponded in size to DhaK1 alone rather than the combined DhaK1-DhaK2.

This fact provides confirmation that dhaK1 and dhaK2 are separate genes in E. coli. Further supporting this contention, the DHA phosphorylation defect in Hfr7::Tn10 could be complemented by the cloned dhaK1 gene alone. Therefore, DhaK1 presumably plays a direct role in DHA phosphorylation, possibly functioning together with DhaK2 as a DHA kinase.

These observations contrast with the earlier report of Jin & Lin (4) suggesting that the PTS is responsible for phosphorylation of DHA. Indeed the region implicated in DHA phosphorylation lacks any apparent Enzyme II of the PTS. However, we were unable to demonstrate phosphorylation of DhaK1 by phosphorylated DhaH in the presence or absence of DHA. Possibly, DhaK2 is the target of phosphorylation.


