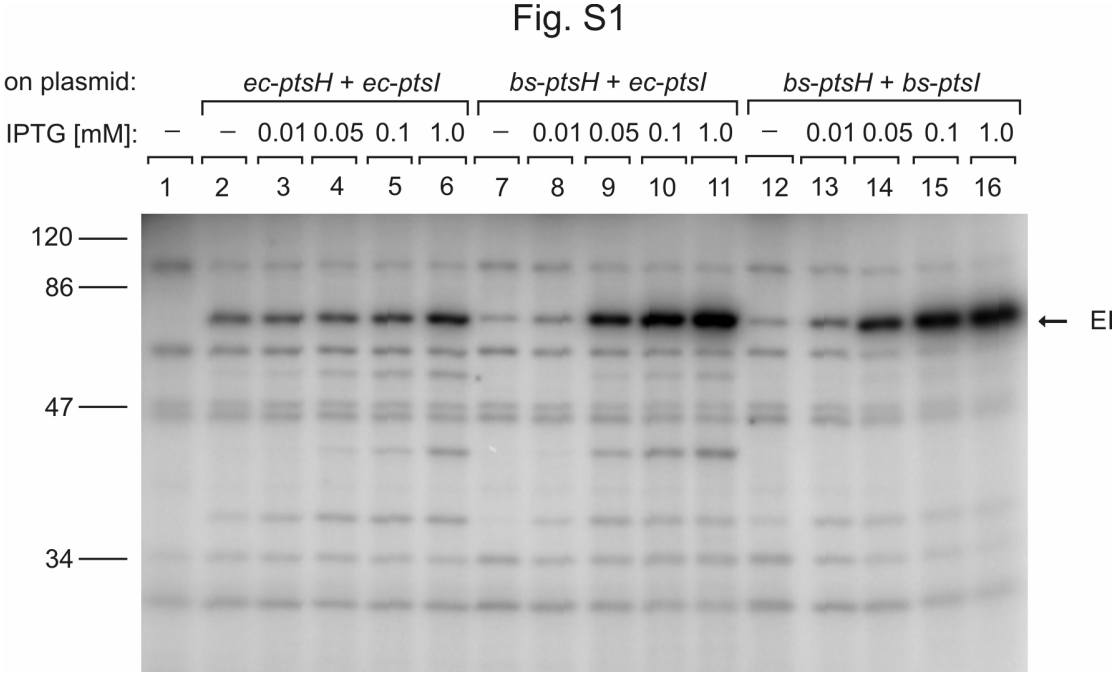
Supplemental material for:

Birte Reichenbach, Daniel A. Breustedt, Jörg Stülke, Bodo Rak and Boris Görke (2007), **"Genetic Dissection of Specificity Determinants in the Interaction of HPr with Enzymes II of the bacterial Phosphoenolpyruvate:Sugar Phosphotransferase System in *Escherichia coli*"** submitted to *J. Bacteriol.*

Enzyme I of *B. subtilis* efficiently autophosphorylates in *E. coli*

It has been speculated that the autophosphorylation rate of EI in *E. coli* could be subject to regulatory mechanisms that may involve small metabolite effectors, proteins or varying subcellular distributions (2, 4). Moreover, dimerization of EI of *E. coli* is extremely sensitive to many parameters like Mg^{2+}, PEP, pH or ionic strength (5). Therefore, it could not be ruled out that the activities of the two EI proteins differ inside the *E. coli* cell. To investigate this possibility, *in vivo* protein phosphorylation assays were performed. The transformants used for the growth tests (Table III) were labeled with $H_3[^{32}P]O_4$ in the presence of different IPTG concentrations and the phosphorylated proteins were separated by SDS-polyacrylamide gel-electrophoresis and detected by phospho-imaging (Fig. S1). In all three transformants the addition of increasing concentrations of IPTG resulted in increasing amounts of phosphorylated EI. As expected from their molecular masses, the EI proteins of *E. coli* and *B. subtilis* (MW= 63.4 kD and 62.9 kD, respectively) migrated at almost identical positions in the gel (Fig. S1; compare lanes 12-16 with lanes 2-11). No significant differences in signal intensities between *E. coli* EI and *B. subtilis* EI were detectable when they were co-synthesized together with *B. subtilis* HPr (Fig. S1; compare the corresponding IPTG concentrations of lanes 7-11 and 12-16). The transformant that synthesized both proteins from *E. coli* displayed a lower amount of phospho-EI at high expression levels when compared with the other two transformants (Fig. S1, compare lanes 2-6 with lanes 7-11 and 12-16). This effect might be attributable to a more efficient drain of the phosphoryl-groups via HPr towards other proteins of the PTS present in the cell.



Legend to Supplemental Figure S1

Fig. S1 Autophosphorylation of EI of *B. subtilis* in *E. coli.* The transformants employed in the growth experiments in Table III were grown in the presence of various IPTG concentrations to obtain different expression levels of the *tacOP*-controlled *ptsH* and *ptsI*-genes. Subsequently, the cells were labeled with $H_3[^{32}P]O_4$, the proteins were separated on SDS/12.5 % polyacrylamide gels and the phosphorylated proteins were detected by phospho-imaging. As a control, the untransformed strain R1969 is employed in lane 1. Note that in the transformants expressing *E. coli* EI two additional proteins become also phosphorylated in an IPTG-dependent manner (lanes 2-11). These signals could represent acetate kinase (AckA, MW = 43,29 kD) and another so far unidentified kinase of *E. coli* which can be phosphorylated by *E. coli* EI (1, 2). Note that in the above experiment (Fig. S1) HPr~P could not be detected. Due to its small size of 88 amino acids it comigrates in a bulk of ^{32}P-labeled low molecular compounds at the bottom of these gels.

Supplemental Materials and Methods

Phosphorylation of enzyme I *in vivo*

Overnight cultures grown in LB containing the appropriate antibiotics were diluted to an OD_{600} of 0.15 in the same medium and grown at 37°C to an OD_{600} of ~0.5. IPTG was added as indicated for the induction of *ptsHI* expression and growth was continued for 20 min before the cells were harvested. Subsequently, the cells were incubated in Tris-medium supplemented with glycerol as carbon source and labeled with $H_3[^{32}P]O_4$ for 30 min. as described previously (3).

Supplemental References

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3. **Görke, B., and B. Rak.** 1999. Catabolite control of *Escherichia coli* regulatory protein BglG activity by antagonistically acting phosphorylations. Embo J **18:**3370-9.
4. **Patel, H. V., K. A. Vyas, X. Li, R. Savtchenko, and S. Roseman.** 2004. Subcellular distribution of enzyme I of the *Escherichia coli* phosphoenolpyruvate:glycose phosphotransferase system depends on growth conditions. Proc Natl Acad Sci U S A **101:**17486-91.
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