

## IL-3

### Interaction for Phosphotransfer between N-Terminal Domain of Enzyme I and HPr of *E. coli* Phosphoenolpyruvate: Sugar Phosphotransferase System.

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The interaction between the N-terminal domain of enzyme I (EIN) and the histidine-containing phosphocarrier protein HPr of the *Escherichia coli* phosphoenolpyruvate:sugar phosphotransferase system has been investigated by Isothermal Titration Calorimetry and heteronuclear magnetic resonance spectroscopy. The chemical shift changes of the backbone NH and  $^{15}\text{N}$  resonances of EIN upon complex formation was followed by recording a series of  $^1\text{H}$ - $^{15}\text{N}$  correlation spectra of uniformly  $^{15}\text{N}$ -labeled EIN in the presence of increasing amounts of HPr at natural isotopic abundance. The equilibrium association constant derived from analysis of the titration data obtained with both methods is about  $1.5 \times 10^5 \text{ M}^{-1}$ . By mapping the backbone chemical shift perturbations on the three-dimensional solution structure of EIN, the binding surface of EIN in contact with HPr was identified. A decrease in conformational stability of the amino-terminal domain of enzyme I produced by phosphorylation of the active-site His 189 seems to have the physiological consequence of promoting phosphotransfer to the phosphocarrier protein, HPr.