SI-4-1

Topology of the Regulatory Protein-Protein Interaction for *Escherichia coli* Diauxism

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Sugar transport by some permeases in *E. coli* is allosterically regulated by the phosphorylation state of enzyme IIA^{glc} ($EIIA^{glc}$) of the phospho*enol*pyruvate:sugar phosphotransferase system. The binding is stimulated by transportable substrates (lactose, melibiose, and raffinose), but not by sugars that are not transported (maltose and sucrose). Treatment of lactose permease with *N*-ethylmaleimide (NEM), which blocks ligand binding and transport by alkylating Cys-148, also blocks ligand binding. Preincubation with the substrate analog β-D-galactopyranosyl 1-thio-β-D-galactopyranoside protects both lactose transport and $EIIA^{glc}$ binding against inhibition by NEM. A collection of lactose permease replacement mutants at Cys-148 showed, with the exception of C148V, a good correlation of relative transport activity and $EIIA^{glc}$ binding. The nature of the interaction of $EIIA^{glc}$ with the cytoplasmic face of lactose permease was explored. Lactose permease mutants with polyhistidine insertions in cytoplasmic loops IV/V and VI/VII and periplasmic loop VII/VIII retain transport activity, but do not bind $EIIA^{glc}$, indicating that these regions of lactose permease may be involved in recognition of $EIIA^{glc}$. Taken together, these results suggest that interaction of lactose permease with substrate promotes a conformational change that brings several cytoplasmic loops into an arrangement optimal for interaction with the regulatory protein, $EIIA^{glc}$. A topological map of the proposed interaction is presented.

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Properties of the Flavoenzyme Pyranose Oxidase from *Oxyporus* sp.

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Pyranose oxidase contained 4 mol of covalently bound FAD per mol of tetrameric protein. The FAD moiety was shown to be covalently linked via the 8α-methylene group to the *N*(1) position of the imidazole ring of histidine. The flavin portion of pyranose oxidase underwent characteristic spectral changes upon changing the pH. The pK_a for the spectral change of the first absorption band was 5.66. Aromatic carboxylates such as 2-hydroxybenzoate and *m*-hydroxybenzoate showed a marked inhibition effect on the enzyme activity by the formation of complex with the active site. The pH dependence of the absorption spectral changes suggested that an ionizable group of a pK_a of 6.4 is involved in formation of complex with 2-hydroxybenzoate. The ionizable group having the same pK_a value was shown to be involved in formation of complex with acetate which had some inhibition effect. The reaction product for D-glucose, the most preferred substrate, was D-*arabino*-hexos-2-ulose. Pyranose oxidase oxidized certain aldonic acid lactones such as L-gulono-1,4-lactone and D-arabinono-1,4-lactone to produce L-ascorbic acid and D-erythroascorbic acid respectively.