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REGULATION OF STYRENE CATABOLISM IN *Pseudomonas fluorescens* ST: FUNCTIONAL CHARACTERIZATION OF THE styS SENSOR KINASE

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Abstract

The regulation of styrene catabolism in *Pseudomonas fluorescens* ST represents a model to understand how the expression of a peripheral catabolic pathway for the degradation of a toxic compound could be integrated into the central metabolism.
In *P. fluorescens* ST the expression of the styrene degradation operon, *styABCDE*, is induced in the presence of styrene and repressed by the simultaneous presence of preferential carbon sources, such as glucose. The promoter of *styABCDE*, P*styA*, is under the control of a two-component system consisting of the hybrid histidine kinase (HK), StyS, and its cognate response regulator (RR), StyR. According to the current model, *styABCDE* styrene-induced transcription is repressed when cells accumulate high levels of phosphorylated StyR (StyR-P), as it occurs in the presence of glucose. The levels of StyR-P are modulated by StyS. StyS contains two putative PAS sensing domains and two complete HK modules. This structural complexity leads to the hypothesis that StyS could integrate two different stimuli, one related to the presence of styrene and a second one representative of the energetic state of the cell, thus determining adequate levels of StyR-P and, ultimately, P*styA* activity.
This study represents a first step toward the structural and functional characterization of StyS and of its interaction with StyR.
In vitro autophosphorylation assays have been performed on the full-length StyS protein and on each isolated HK domains (N-terminal HK1D and C-terminal DHK2, respectively). Results showed that styrene exerts a positive effect on the phosphorylation activity of StyS and HK1D by decreasing the dephosphorylation rate of the kinase, and pointed out that styrene is directly perceived by the *N*-terminal PAS-1 domain. Consistently with the experimental failure of DHK2 to autophosphorylate *in vitro*, we propose that the phosphoryl group is provided to the *C*-terminal domain by the *N*-terminal histidine kinase, through an internal phosphorelay mechanism. Transphosphorylation assays demonstrated that StyS is able to transfer a phosphoryl group to StyR, most likely through the C-terminal kinase domain.
Therefore, the reduced dephosphorylation rate observed for HK1D, with respect to the full-lenght StyS, strongly suggests that the C-terminal kinase domain may play a main role in modulating the phosphorelay rate, hence in determining the levels of StyR-P. In this view, the activity of StyS could be determined by a still unknown stimulus, perceived by the PAS domain of HK2 and probably related to the redox status of the cell, in accordance with our current model of styrene-catabolism regulation.