

Towards a Quantitative Description of the EnvZ/OmpR Two-Component System During Osmotic Signaling

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I. PURPOSE

In bacteria, the paradigm for signal transduction is the two-component regulatory system. The first component is a sensor kinase, usually a membrane protein that is phosphorylated by ATP on a conserved histidine residue. The response regulator receives the phosphoryl group from the kinase and modifies its response, which is most often a change in gene expression (3). The EnvZ/OmpR two-component regulatory system is best known for regulating the porin genes *ompF* and *ompC* in response to changes in the osmolality of the growth medium (7). Phosphorylation of OmpR increases its affinity for the regulatory regions of the porin genes, altering their expression (2, 4). Phosphorylation also alters the interaction with EnvZ and OmpR (6). N-terminal phosphorylation of OmpR stimulates C-terminal DNA binding and this communication between domains is bi-directional i.e., DNA binding stimulates phosphorylation (1). We proposed a four-state model for the steps involved in OmpR/DNA binding and proposed that OmpR is phosphorylated by the kinase EnvZ while bound to DNA (1). Experiments are in progress to characterize this EnvZ/OmpR/DNA complex. Our laboratory is interested in a molecular and quantitative view of the signal transduction process, e.g. what is the signal that EnvZ senses and how does signaling affect the interactions of EnvZ with OmpR and OmpR with DNA?

II. SUMMARY OF RESULTS

In order to study EnvZ/OmpR interactions, we employed a full-length EnvZ chimera fused to GFP that was over-expressed and targeted to the inner membrane. Spheroplasts were prepared and lysed in microtiter plates containing purified, fluorescent-labeled OmpR protein. Fluorescence resonance energy transfer (FRET) from the GFP donor to fluorescein- or rhodamine-conjugated

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OmpR acceptor occurred, indicating that the two proteins interact (5). We then used FRET to further characterize the effect of phosphorylation, DNA binding and osmotic stress on the interaction parameters.

III. CONCLUSIONS

Our results indicate that phospho-OmpR protein (OmpR~P) has a reduced affinity for the EnvZ kinase compared to the unphosphorylated protein (5). Two-component systems have been described as robust, i.e., the level of OmpR~P is not sensitive to alterations in the levels of EnvZ and OmpR. Recent results that are not consistent with this view will be presented.

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