

Characterizing Response Regulator Activation by MALS

All organisms have molecular mechanisms in place to sense and respond to environmental changes. Bacteria use two-component signal transduction pathways, which comprise a histidine kinase (HK) and a response regulator protein (RR). Extracellular stimuli sensed by the HK induce an auto-phosphorylation reaction at a histidine residue of the enzyme that is subsequently transferred to a specific aspartate residue at the receiver domain of a cognate RR. Upon phosphorylation, the output domain of the RR is activated, leading to changes in gene expression, protein-protein interactions or enzymatic activities.

Although all receiver domains of RR share a highly conserved protein fold, the conformational or oligomerization state changes that happen upon protein phosphorylation may differ among these proteins. Our initial goal was the characterization of the oligomeric transitions between inactive and active states of two RRs (R1 and R10), which are phosphorylated by the same HK.

We used an SEC-MALS system consisting of an analytical size exclusion gel chromatography column (Superdex 10/300 GL) coupled to a light scattering detector (miniDAWN TREOS) and differential refractive index detector (Optilab rEX).

Our data show that phosphorylation of both proteins leads to drastic changes in their molecular conformation. For R1 (Fig. 1), the protein behaves as a monomer in both situations ($M_w \sim 14$ kDa). However, the difference in the elution volumes for each state (inactive ~ 10.7 mL; active ~ 12.8 mL) indicates a remarkable change in the overall shape from a loose to a compact conformation when the protein is phosphorylated. On the other hand, R10 activation is followed by a dimerization event ($M_{w_{inactive}}: 26.0$ kDa, $M_{w_{active}}: 51$ kDa) (Fig. 2). Once R10 has a transcription factor in its output domain; dimerization would favor interactions with DNA as observed in other protein homologues.

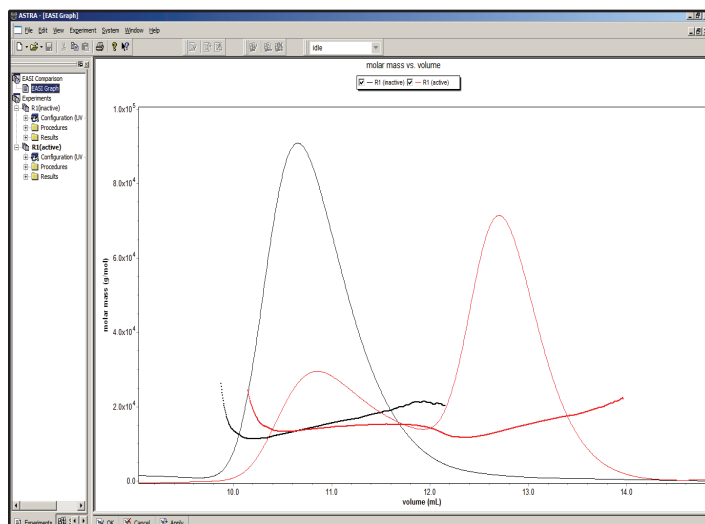


Figure 1. Effect of phosphorylation in the conformation of R1. Upon phosphorylation, the protein undergoes a drastic change in its overall shape as evidenced by the change in the elution volume and no alteration in the molar mass. Data show clearly that phosphorylation of the protein favors the formation of a more compact structure when compared to the inactive state. It can also be observed that a fraction of R1-P lost its phosphate returning to the inactive state, as showed by the red left peak around 11 mL.

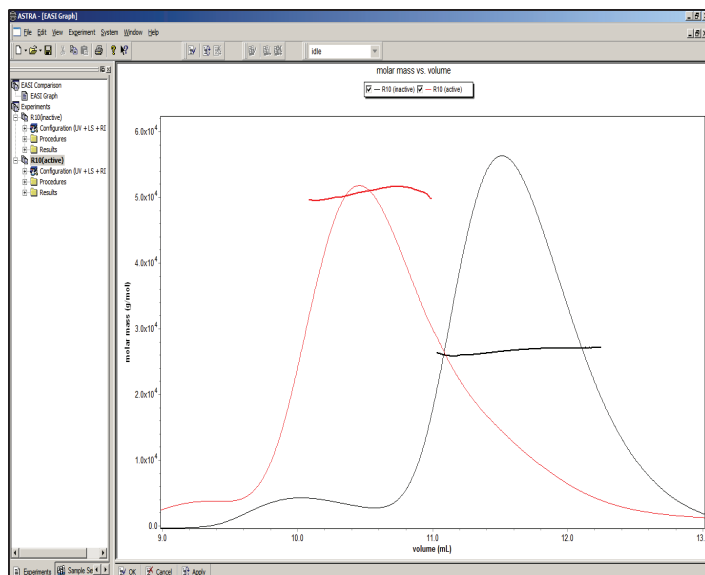
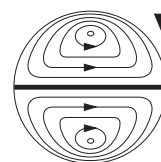


Figure 2. Effect of phosphorylation in the oligomeric state of R10. Phosphorylation of R10 leads to the formation of a protein dimer form.

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