Interplay between reaction stoichiometry and effective concentration: A structure-based synergistic study on Grb2:Sos1 complex.

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***Short Abstract* — Forming multivalent complexes by combining relatively weak promiscuous interactions is a strategy that is often used to increase the affinity and specificity of biomolecular complex formation among signaling proteins. However, often there is no clear understanding of the structural details by which the stoichiometry and the distribution of these complexes are affected by multivalent interactions between signaling protein partners. Growth receptor bound protein-2 (Grb2) is an adaptor protein that mediates the recruitment of the nucleotide exchange factor Son of Sevenless-1 (Sos1) from the cytosol to the plasma membrane where it activates Ras by inducing the exchange of GDP for GTP. Grb2 is composed of two SH3 domains that can form complexes with multiple polyproline motifs on Sos1. The stoichiometry of the Grb2:Sos1 complex is poorly understood. In this work, we investigate the formation of Grb2:Sos1 complexes at multiple resolutions. First, we examine complex formation between the individual polyproline peptides of Sos1 and the SH3 domains of Grb2 using a combination of evolutionary information and binding energy calculations. Secondly, we considered a Grb2 with one of its SH3 domains bound to Sos1. Then, using a hybrid approach involving molecular dynamics simulations and polymer models, we estimate the enhancement in local concentration of a polyproline motif on Sos1 near the unbound SH3 domain of Grb2. This allows us to calculate the intramoleclular equilibrium constant for the crosslinking of Grb2 on SOS1. Finally, we use thermodynamic modeling to calculate the stoichiometry and predict the distribution of the complexes that are formed at physiological concentrations of the signaling proteins. This is the first such systematic analysis involving the synergistic combination of sequence, structure, and dynamic analyses to determine the stoichiometry of the complexes that are dominant in the cellular environment.**

***Keywords* — Multivalency, effective concentration, intramolecular binding, stoichiometry, adaptor protein.**

I. Background

Numerous signaling proteins use multivalent binding to increase the specificity and affinity of their interactions within the cell. Such an enhancement arises because the effective binding constant for multivalent binding is larger than the binding constants for each individual interaction. This strategy is wide spread with signaling proteins exhibiting a variety of combinations of domains (modules), PH, PTB, SH2, SH3, etc., that allow them to attach to one or more proteins at multiple sites [1]. The evolution of such modularity has played an important role in the development of signaling pathways in eukaryotes [2].

We have carried out a theoretical study of the binding of the widely expressed adaptor protein Grb2, through its SH3 domains, to the nuclear exchange factor Sos1. Grb2 contains one SH2 domain flanked on each side by a SH3 domain [3]. The activation of the Ras signaling pathway requires the recruitment of SOS1 from the cytosol to the plasma member where it activates Ras by inducing the exchange of GDP for GTP [4]. This recruitment is mediated by Grb2, which couples Sos1 to phosphorylated scaffolding proteins that contain sequences of the binding motif for the Grb2 SH2 domain, YXNX. The equilibrium constants for both the N-SH3 and the C-SH3 domains of Grb2, binding to four peptides in Sos1, have been determined experimentally [5].

II. Results And Conclusion

The theoretical study we present represents a first systematic analysis that combines sequence comparison, structure, molecular dynamics simulations and polymer models to determine the stoichiometry of the complexes that dominate the cellular environment. In the approach presented here, a combination of evolutionary analysis of the sequences and binding energy calculations is used to predict the presence of a new binding motif for Grb2 in Sos1. Secondly, polymer theory is used in combination with molecular dynamics (MD) simulations to calculate the enhancement in binding constants due to local concentration where both the flexibility of the modular protein and the disordered region containing binding motifs are taken into account. We conclude with a detailed evaluation of the formation of Grb2:Sos1 complexes under physiological conditions and discuss the implications for cell signaling. The approach developed here has applicability beyond the current implementation and provides a general framework for handling the multivalency of protein-protein interactions where disordered regions play a significant role.

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