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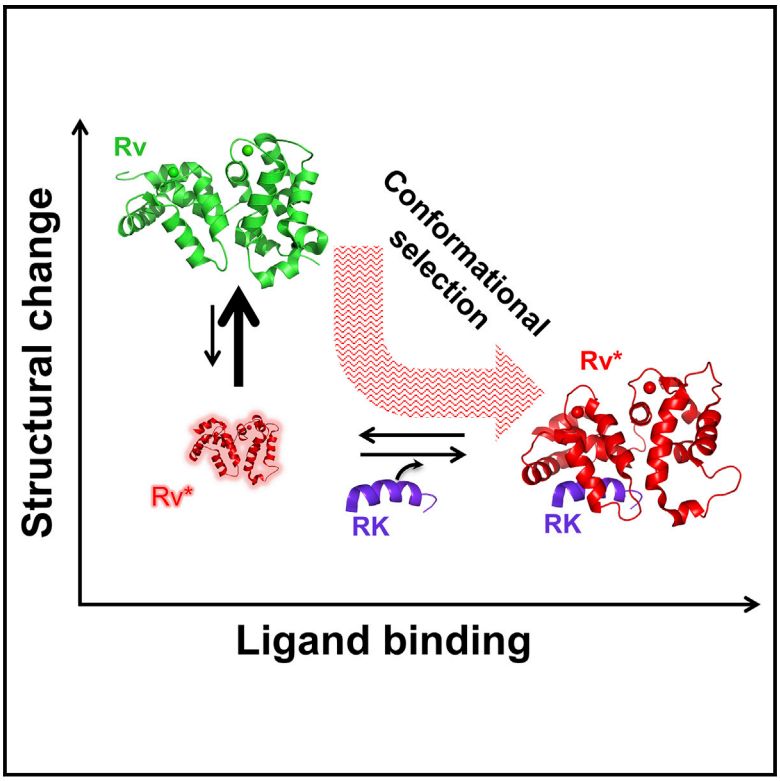
Conformational Selection in a Protein-Protein Interaction Revealed by Dynamic Pathway Analysis

Graphical Abstract



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In Brief

Despite the central role of protein/protein interactions in biological processes, two fundamentally opposite binding mechanisms have been debated: conformational selection versus induced fit. Using NMR and pre-steady-state kinetics, Chakrabarti et al. quantitatively show that recoverin binds rhodopsin kinase exclusively via conformational selection.

Highlights

- Recoverin binds rhodopsin kinase by conformational selection
- NMR and stopped flow kinetics reveal binding mechanism
- Direct experimental distinction between conformational selection versus induced fit
- Binding-competent state with exposed hydrophobic binding pocket is only 3% populated

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Conformational Selection in a Protein-Protein Interaction Revealed by Dynamic Pathway Analysis

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SUMMARY

Molecular recognition plays a central role in biology, and protein dynamics has been acknowledged to be important in this process. However, it is highly debated whether conformational changes happen before ligand binding to produce a binding-competent state (conformational selection) or are caused in response to ligand binding (induced fit). Proposals for both mechanisms in protein/protein recognition have been primarily based on structural arguments. However, the distinction between them is a question of the probabilities of going via these two opposing pathways. Here, we present a direct demonstration of exclusive conformational selection in protein/protein recognition by measuring the flux for rhodopsin kinase binding to its regulator recoverin, an important molecular recognition in the vision system. Using nuclear magnetic resonance (NMR) spectroscopy, stopped-flow kinetics, and isothermal titration calorimetry, we show that recoverin populates a minor conformation in solution that exposes a hydrophobic binding pocket responsible for binding rhodopsin kinase. Protein dynamics in free recoverin limits the overall rate of binding.

INTRODUCTION

Molecular recognition dynamics in protein/ligand or protein/protein interactions is a fundamental phenomenon that has been extensively discussed during the last 50 years in light of two opposing mechanisms: the induced fit (IF) (Koshland, 1958) and the conformational selection (CS) model (Changeux and Edelstein, 2011; Monod et al., 1965). The experimental detection of discrete conformational sub-states of individual proteins in solution and their structural characterization has brought renewed interest to the CS model in recent years (Boehr et al., 2006; Clore, 2014; Cornish-Bowden, 2014; Di Cera, 2014; Feixas et al., 2014; Hatzakis, 2014; James et al., 2003; James and Tawfik, 2005; Lange et al., 2008; Nussinov et al., 2014; Tzeng and Kalodimos, 2009; Vogt et al., 2014). However, even in cases where atomic resolution structures of different conformations of the free protein have been solved (James et al., 2003; Lange et al., 2008), it is not clear whether or not this conformational equilibrium is important for ligand binding. Sampling a "bound-like conformation" of the protein before the ligand is actually bound has been used as the strongest evidence for CS in a growing number of systems (Boehr et al., 2009; James and Tawfik, 2005; Lange et al., 2008; Tzeng and Kalodimos, 2009). However, pre-sampling the bound conformation is a necessary but not sufficient condition for a CS mechanism (Bouvignies et al., 2011; Tang et al., 2007; Weikl and Paul, 2014). The distinction between the two opposing binding models can only be made on the basis of flux measurements through the two pathways based on simple and long-known kinetic principles (Fersht, 1999; Foote and Milstein, 1994; James et al., 2003; Lancet and Pecht, 1976; Monod et al., 1965; Strickland et al., 1975). Fueled by an explosion of publications claiming to reveal a CS mechanism based only on pre-existing structures in the apo-proteins (Al-Hashimi, 2013; Boehr et al., 2006; James et al., 2003; Lange et al., 2008), several papers appeared reminding the community of the fundamental need to kinetically discriminate between the two models (Daniels et al., 2014; Greives and Zhou, 2014; Hammes et al., 2009; Weikl and Paul, 2014; Zhou, 2010).

Previous studies have focused solely on either a structural argument or a kinetic determination of flux (Foote and Milstein, 1994; Lancet and Pecht, 1976), but not on both as needed (Hammes et al., 2009; Palmer, 2014). Here, we combine both