

Structural Studies of the Activation Mechanisms of Arrestin

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Beamline(s): X25

The G-protein coupled receptor signal pathway is one of the most important signaling pathways in eukaryotic organisms. It is directly responsible for the perception of light and smell, heart beat regulation, hormone action and signal transduction by various neurotransmitters. All G-protein coupled receptors (GPCR) are integral membrane proteins, which share a common structural motif of seven transmembrane helices. Upon receiving a signal, the receptor activates an enzymatic cascade starting with the G-protein which activates further effectors downstream. The cascade is terminated by the combined action of a specific kinase which phosphorylates the activated receptor and a class of proteins called arrestins that subsequently bind to the phosphorylated receptor and inhibit further interaction with the G-protein.

Recently our group solved the structure of visual arrestin (Hirsch *et al.*, 1999, Cell 97, p257), which is expressed only in the retina and pineal gland and interacts with its bona fide partner rhodopsin (the visual receptor). β -arrestins (β -arrestin1 and β -arrestin2), on the other hand, are ubiquitously expressed in tissue and bind to a variety of GPCRs, like the adrenergic and muscarinic receptors. Binding of β -arrestins is also an important secondary signal. It has been shown that β -arrestins function as adaptor proteins mediating endocytosis of GPCRs via clathrin coated pits. It was also shown that β -arrestin binds to the SH3-domain of src kinases in a receptor-activation dependent manner. This interaction is the crucial and long-missing link between the GPCR activation pathway and the mitogen-activated protein (MAP) kinase pathway. More recently, it was also shown that β -arrestin also interacts with N-ethylmaleimide-sensitive fusion protein (NSF), therefore linking receptor sequestration and vesicular transport. Increasing evidence suggests that arrestins plays a pivotal role in linking vital cellular signaling pathways, making it likely that more interactive partners of non-visual arrestins will be discovered in the near future.

Our aim is to understand the molecular details responsible for the specificity of β -arrestins towards their receptors and their interactions with various partner proteins.

We have solved two crystal forms of a truncated form of bovine β -arrestin1, which lacks the last 25 amino acids but retains all of the biochemical properties of the wild-type protein: a monoclinic crystal form, diffracting to 1.8 Å at X25, was solved by SAD-phasing using selenomethionine substituted protein. A trigonal crystal form diffracting to 2.1 Å at the same beamline was also solved by SAD-phasing at the Se edge on the same beamline. Currently we are in the final stages of the structure refinement.

We have also investigated mutational variants of β -arrestin which adopt an activated conformation after losing the regulatory components which keep the protein in an inactivated state. One or more of these variants should shine light on the underlying activation mechanisms of β -arrestins. We have solved the structure of one of these mutants by molecular replacement using data obtained at X25 and have finished refinement.

We are expecting to have the results from this study published soon.