Solution Conformation and Activation of Visual Arrestin

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The visual cascade consists of the photoactivation of rhodopsin which can then activate transducin, a heterotrimeric G-protein. The activated transducin ultimately produces a nervous signal. A single photoactivated rhodopsin (Rho\textsuperscript{*}) is capable of activating many transducin molecules, and therefore a mechanism is required for rapidly attenuating the ability of Rho\textsuperscript{*} to activate transducin. Rhodopsin kinase multiply phosphorylates the C-terminal tail of Rho\textsuperscript{*}, reducing the ability of Rho\textsuperscript{*} to activate transducin. Arrestin is a soluble protein that completely shuts off signalling by Rho\textsuperscript{*}. Arrestin is present in the cell cytosol at all times, but it only blocks Rho\textsuperscript{*} signalling once the C-terminal tail of rhodopsin becomes phosphorylated. In this respect, arrestin is thought to become "activated" by binding the phosphorylated C-terminus of rhodopsin. Arrestin can also be activated by interaction with a synthetic phosphopeptide in solution: the binary arrestin-phosphopeptide complex is then capable of binding to unphosphorylated Rho\textsuperscript{*}. The phosphopeptide apparently causes changes in the conformation and/or quaternary structure of arrestin that allow it to bind to photoactivated rhodopsin. Various point mutations can also activate arrestin; one of these point mutants, arrestin-R175Q, was used in our SAXS studies. Our aim was to characterize the changes in conformation and/or quaternary structure that accompany "activation" of arrestin and allow it to bind to photoactivated rhodopsin with high affinity.

The forward scattering from solutions of arrestin showed a protein concentration dependence that was consistent with a monomer-dimer equilibrium. This equilibrium was unaffected by the R175Q mutation, and the presence of phosphopeptide had only a minor effect outside of the physiological concentration range. There were no changes in the radius of gyration associated with either binding of the phosphopeptide or the R175Q mutation. To search for changes in conformation, the entire scattering curves for wild-type arrestin, arrestin R175Q, and arrestin in the presence of phosphopeptide, were compared. It was found that there were small changes produced by the phosphopeptide and R175Q mutation in the region from \( s = 0.22 \) nm\(^{-1}\) to \( s = 0.30 \) nm\(^{-1}\). These changes are consistent with localized movements of loops and/or the N- and C-termini, but are not consistent with any large conformational change that could bring the N- and C-terminal domains close together.

In summary, our SAXS results indicate that arrestin participates in a monomer-dimer equilibrium in solution, and the "activation" of arrestin in solution involves only minor conformational changes. These results have raised the possibility that the dimeric form of arrestin may be important for its function. We have used our SAXS data, in combination with the high resolution crystal structure of arrestin (1,2), to identify the structure of the dimer that is present in solution. In the crystal, arrestin was present as a tetramer in the asymmetric unit: the tetramer has a single two-fold axis of symmetry, and can be described as a "dimer of dimers", with two choices for the dimer that is present in solution. In addition, there are two dimers present in the crystal structure that result from crystallographic symmetry operations. On the whole, the crystal structure offers 4 possible choices for the structure of the dimer in solution. Using our SAXS data, one of the dimers was identified as the most likely candidate (Figure 1). Further experiments are underway to confirm the identity of the dimer, and to investigate the possibility that the dimer is the biologically active species.
Figure 1: The arrestin dimer that is present in solution.

References