Chapter 5
Arrestin-3: The Structural Basis of Lower Receptor Selectivity

Benjamin W. Spiller, Xuanzhi Zhan and Vsevolod V. Gurevich

Abstract Arrestin-3 (β-arrestin2) is the second member of the non-visual arrestin subfamily cloned. Together with arrestin-2 (β-arrestin1), it is responsible for arrestin function outside the visual system. Arrestins 2 and 3 are multi-functional proteins, originally identified as important for termination of signaling through G protein-coupled receptors (GPCRs), but now recognized as versatile regulators of signaling and trafficking of most GPCRs, as well as signaling switches controlling the balance between G protein-dependent and -independent GPCR signaling. Arrestin-3 is notably the least selective arrestin family member in that it binds a wide variety of GPCRs and that binding is less dependent on receptor activation and phosphorylation than for other arrestin subtypes. A recently determined arrestin-3 structure reveals that the basal conformation of arrestin-3 is similar to other arrestins, and that similar molecular interactions stabilize the basal state in all arrestins. A disruption of a structural element in the C-domain of arrestin-3 has been implicated as the cause of the reduced selectivity of arrestin-3. Here we compare the basal arrestin-3 structure with the recently determined structure of an activated receptor-bound arrestin-1.

Keywords Arrestin · Arrestin-3 · GPCR binding · Receptor specificity · Crystal structure · Receptor selectivity

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Introduction

The four arrestin subtypes expressed in mammals can be broadly classified into visual and non-visual groups (Gurevich and Gurevich 2006a). The visual arrestins, arrestin-1 (rod arrestin) and arrestin-4 (cone arrestin) are expressed only in photoreceptors and bind rod and cone opsins (Strissel et al. 2006; Hanson et al. 2007; Nikonov et al. 2008). In contrast, arrestin-2 and arrestin-3 (also referred to as β-arrestin1 and β-arrestin2, respectively) are present in most cells, and interact with hundreds of G protein-coupled receptors (GPCRs) (Gurevich and Gurevich 2006b). In most cell types arrestin-2 is the major arrestin family member, being more abundant than arrestin-3 by ∼10–20 fold (Gurevich et al. 2002, 2004). In addition to their roles in terminating GPCR signaling, arrestins 2 and 3 also serve as clathrin adaptors, thereby controlling GPCR endocytosis (Laporte et al. 1999; Oakley et al. 1999; Goodman et al. 1996), and as signaling hubs, where they scaffold and direct kinase networks, and as mediating ubiquitination of receptors and other signaling molecules [reviewed in Gurevich and Gurevich (2006b), DeWire et al. (2007), Jean-Charles et al. (2016)]. A key functional difference between arrestin-3 and other family members is its relative receptor promiscuity. Binding studies comparing different arrestins to different functional states of their cognate receptors (active phosphorylated, inactive phosphorylated, active unphosphorylated, and inactive unphosphorylated) revealed arrestin 3 to be significantly less specific than other arrestins. Arrestin-1 is the most specific and shows a >10 fold preference for active, phosphorylated, receptor over inactive, phosphorylated, receptor (Gurevich et al. 1993; Gurevich 1998). Both arrestin-2 and arrestin-3 are less selective, and show an ∼2 fold preference for active, phosphorylated, receptor over inactive, phosphorylated, receptor, with arrestin-3 being the least selective (Gurevich et al. 1995). The requirement for receptor phosphorylation can be more effectively relieved through mutagenesis in arrestin-3 than in other arrestins, further supporting the conclusion that arrestin-3 is the most promiscuous arrestin (Gurevich and Gurevich 2006b).

In addition to being more promiscuous, arrestin-3 generally shows higher affinity for GPCRs, higher affinity for clathrin, as well as unique kinase scaffolding activity (Gurevich and Gurevich 2006b; Goodman et al. 1996; DeWire et al. 2007; Kohout et al. 2001; Oakley et al. 2000). Importantly, only arrestin-3, but not highly homologous arrestin-2, facilitates the activation of JNK family kinases (McDonald et al. 2000; Song et al. 2009a; Miller et al. 2001; Breitman et al. 2012; Zhan et al. 2011a, 2013, 2016; Kook et al. 2013). Despite these unique features, the two proteins are partially redundant because knockout a either non-visual arrestin is tolerated, but the double knockout is embryonic lethal (Kohout et al. 2001).

Arrestin 3 was the final isoform to have its structre reported, with arrestin 1, 2, and 4 published previously (Hirsch et al. 1999; Han et al. 2001; Kang et al. 2009; Milano et al. 2002, 2006; Sutton et al. 2005). Here we review the arrestin-3 structure, with the added benefit of comparison to the recent structure of the rhodopsin-arrestin-1 complex, which offers the first insight into an activated

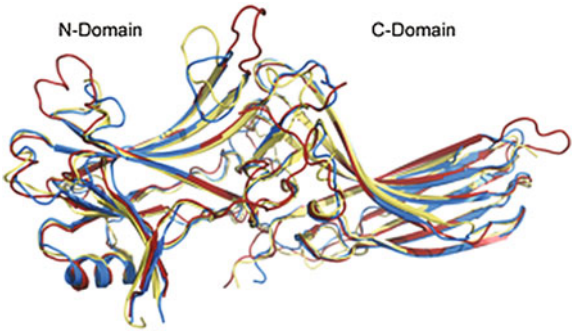

Fig. 5.1 Arrestin 3 is structurally similar to Arrestins 1 and 2. Arrestin 3 (pdb ID 3pd2) is shown in *blue*, Arrestin 1 (pdb ID 1cf1) is shown in *red*, and Arrestin 2 (pdb ID 1cf1) is shown in *yellow*. Arrestins are shown as a *ribbon diagram*, highlighting the conservation of all secondary structural elements

receptor-bound arrestin. The overall fold of arrestin-3 resembles that of the other family members, Fig. 5.1. The key features known to stabilize the basal state, the three-element interaction and the polar core, are present in the now expected form (Fig. 5.2), but the structure identified certain peculiarities characteristic for this subtype.

Arrestin-3 Structure

As expected, the arrestin-3 adopts a canonical arrestin fold (Figs. 5.1 and 5.2). It is composed of structurally homologous domains, the N-terminal and C-terminal domains, each of which is formed by a 7-stranded beta sandwich. The two domains are connected by a flexible linker and stabilized by inter-domain contacts. The extreme carboxy terminus traverses the C-domain to form critical interactions with the N-domain (three-element interaction, Fig. 5.2b). The last residue visible in the arrestin-3 structure is the arginine in the C-terminus that contributes a salt bridge to a critical functional component, the phosphate sensor (polar core, Fig. 5.2c). Additionally, three residues on this C-terminal peptide form part of the three-element interaction.

Intermolecular interactions hold arrestins in their basal conformations. Two sets of interactions known to be critical are the three-element interaction and the polar core (Fig. 5.2). Disruption of either of these interactions in known to reduce fidelity in the arrestin-receptor interaction, likely by decreasing the activation barrier for the transition to the activated state (Gurevich and Gurevich 2006b; Kovoor et al. 1999; Celver et al. 2002; Song et al. 2009b; Gurevich et al. 2011). The arrestin-3 structure confirmed that alterations to the polar core are not responsible for the increased promiscuity of arrestin-3. The polar core in arrestin-3 includes identical residues in

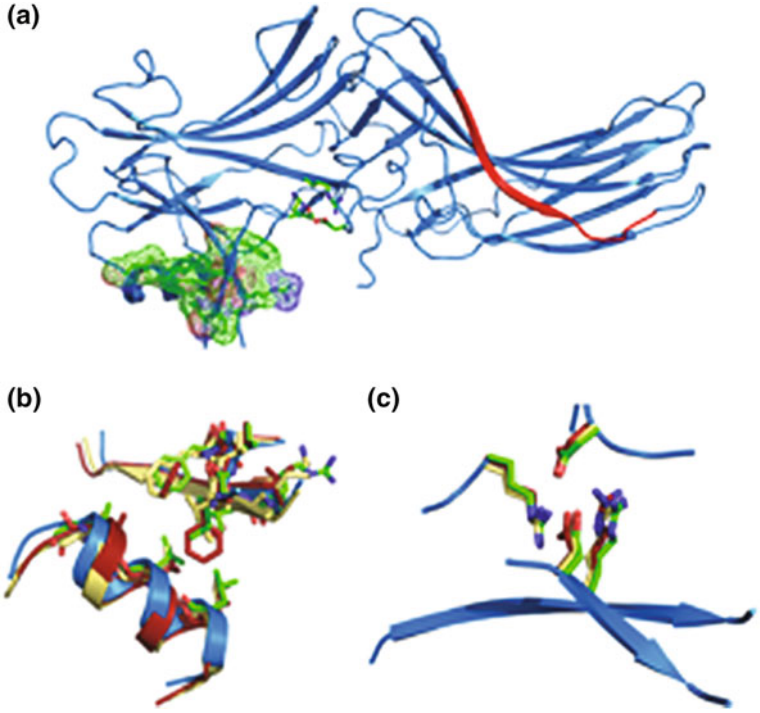

Fig. 5.2 Structurally important features are conserved in Arrestin 3. **a** Arrestin 3 with the conserved three-element interaction highlighted as a Van der Waals mesh and the polar core side chains rendered as stick figures. Additionally, the disrupted beta strand in the C-domain of Arrestin 3 is colored *red*. **b** and **c** Expanded overlays of Arrestins 1, 2, and 3, colored as in Fig. 5.1 and rendered from the coordinates listed in Fig. 5.1. Both views are rotated ~90° toward the reader, about a horizontal axis, relative to the view in **a**. **b** Conserved three-element interaction within three arrestins. **c** Near perfect conservation of the polar core among arrestins 1, 2, and 3. **b** Outermost strand of the arrestin 3 C-domain is also distorted relative to arrestin 2. The disruption is small, ~2 Å, but it disrupts hydrogen bonding to the adjacent strand and likely reduces the energy barrier to activation

nearly identical orientations to those observed in arrestin-1 and arrestin-2 (Fig. 5.2c). The polar core is essentially unchanged in arrestin-3, indicating that a different structural feature is responsible for the unique functional characteristics of arrestin-3.

The three-element interaction, although somewhat less well studied, is thought to serve as a molecular clamp that prevents release of the C-tail (Gurevich 1998; Vishnivetskiy et al. 2000). Release of the C-tail is a necessary step in arrestin activation (Palczewski et al. 1991; Kang et al. 2015; Shukla et al. 2013). In arrestin-3, the three-element interaction is present, but it is altered, as compared to arrestin-1 in three ways. The interaction involves three hydrophobic residues on each of three different secondary structural elements: β-strand I contributes Val8,

Ile9, and Phe10; α-helix I contributes Leu101, Leu105, and Leu109; and the C-tail contributes Phe386, Val387, and Phe388 (bovine arrestin-1 numbering). In arrestin-3, Val8 is replaced with an Arg, Ile9 is replaced with a Val, and Phe386 is replaced with an Ile (Fig. 5.2b). The possibility that these mutations may result in the C-tail being more weakly held in arrestin-3 than in arrestin-1 has not been tested experimentally. In any event, this change is likely not responsible for the entirety of the differences between the arrestins because arrestin-2 and arrestin-3 have identical three-element interactions (Fig. 5.2b), indicating another structural features must underlie the functional differences between the two non-visual arrestins.

An additional difference between arrestin-3 and arrestin-2 is a disruption in the packing of the C-domain. Residues 257–260, which form part of the outer most "strand" in the C-domain, are displaced relative to arrestin-2. The displacements are relatively small (1.3–2.0 Å), but are large enough to disrupt the β-sheet hydrogen bonding pattern, resulting in the loss of 2 hydrogen bonds (Zhan et al. 2011b). Despite the relatively small size of the distortion, it seems important for three reasons: it is substantially larger than the mean coordinate error of the structure; it occurs in a region known to be important for receptor binding; and the C-domain (including this strand) is essentially invariant in all arrestin structures (Hirsch et al. 1999; Han et al. 2001; Kang et al. 2009; Milano et al. 2002, 2006; Zhan et al. 2011b; Vishnivetskiy et al. 2004).

The importance of this distortion was tested by exchanging the strand with the homologous strand from arrestin-2. A bioluminescence resonance energy transfer-based arrestin recruitment experiment designed to determine the fraction of receptor-bound arrestin in the cell revealed that substitution of the distorted strand into arrestin-2 conferred arrestin-3 like features on arrestin-2 (Zhan et al. 2011b). β2AR binding of these chimeras and the wild type arrestins was compared in an arrestin recruitment assay (Zhan et al. 2011b). This assay revealed that distortion of the C-domain sheet promoted binding to inactive and active β2AR (Zhan et al. 2011b), a hallmark feature of arrestin-3 (Gurevich et al. 1995; Kovoor et al. 1999; Celver et al. 2002). Conversely, the introduction of the arrestin-2 residues into arrestin-3 reduced binding to unstimulated β2AR (Zhan et al. 2011b).

Comparisons to the Rhodopsin-Arrestin-1 Complex

The recently determined structure of rhodopsin bound to arrestin-1 (Kang et al. 2015) allows analysis of how differences in the three-element interaction and the disrupted β-strand in the C-domain might be important for the conformational changes associated with receptor binding. Figure 5.3 shows a comparison of receptor-bound arrestin-1 and the basal structure of arrestin-3. The most significant changes are the re-structuring of the finger loop (residues 64–77), the release of the C-tail from the N-domain, and the rotation of the C-domain relative to the N-domain. The finger loop intercalates into the rhodopsin, fitting into the cavity between the cytoplasmic ends of the transmemebrane helices that opens upon

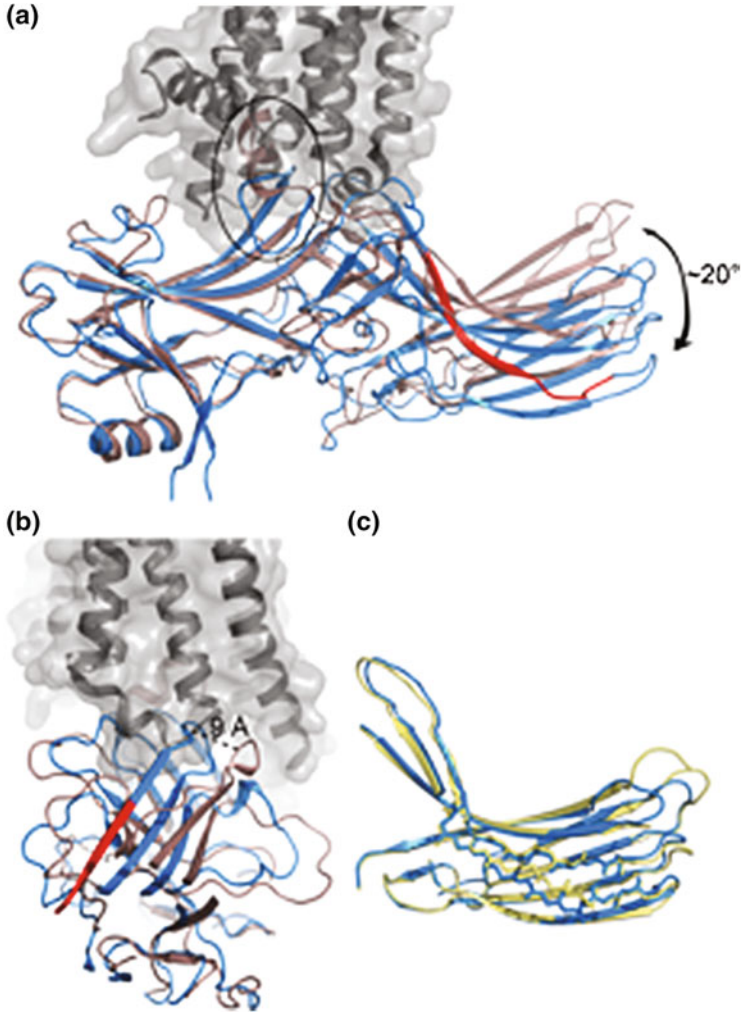

Fig. 5.3 Arrestin 3 is configured to transition to the receptor bound state. **a** Structural rearrangements that occur upon receptor binding. Arrestin 1 shown in salmon undergoes significant conformational changes upon rhodopsin (*grey*) binding (the arrestin-rhodopsin complex is rendered from (pdb 4zwj). The finger loop, highlighted with a *black ellipse*, undergoes a loop to helix transition and inserts into the helical bundle of the receptor. The C-domain rotates $\sim 20°$ relative to the N-domain. The arrestin 3 structure, overlayed based on an alignment to the N-domain, is shown in *blue*, with the outermost strand of the C-domain colored *red*. The homologous strand in arrestin 1 undergoes an ~ 9 Å displacement upon activation, as shown in **b**, in which the view is rotated 90° toward the reader about a vertical axis **c** an overlay of arrestin-1, yellow, with arrestin-3, blue, showing the connection between the finger loop and the loosely packed outer strand in arrestin-3. Loose packing of this strand may accomadate the conformational change associated with activation

GPCR activation (Farrens et al. 1996). This interaction, however, does not appear to require a substantial conformation change on the part of arrestin. The finger loop is the loop between the 4th and 5th strands of the N-domain and is structurally variable in arrestins. An interdomain conformational change is not likely required for it to adopt the active conformation.

The rotation of the C-domain by $\sim 20°$, in contrast, requires a complete reorganization of the interdomain interface and a coincident translation of the beta-sandwich. In arrestin-1, this reorganization results in a repacking of the outermost strand, such that it forms beta strand hydrogen bonds with the neighboring strand for only 7 residues, as compared to 11 residues in the basal state. If arrestin-3 undergoes a similar conformational change upon receptor binding, the loose packing of this strand in the basal state may better accommodate the conformational change associated with activation (Fig. 5.3c). This would explain apparently lower energy barrier, which results in high binding to inactive phosphorylated receptor and wide variety of GPCRs.

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