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SAXS and X-ray Crystallography Suggest an Unfolding Model for the GDP/GTP Conformational Switch of the Small GTPase Arf6

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The small GTPases Arf1 and Arf6 have nonoverlapping functions in cellular traffic despite their very high sequence and structural resemblance. Notably, the exquisite isoform specificity of their guanine nucleotide exchange factors and their distinctive sensitivity to the drug brefeldin A cannot be explained by any straightforward structural model. Here we integrated structural and spectroscopic methods to address this issue using Δ13Arf6-GDP, a truncated mutant that mimics membrane-bound Arf6-GDP. The crystal structure of Δ13Arf6-GDP reveals an unprecedented unfolding of the GTPase core β-strands, which is fully accounted for by small-angle X-ray scattering data in solution and by *ab initio* three-dimensional envelope calculation. NMR chemical shifts identify this structural disorder in Δ13Arf6-GDP, but not in the closely related Δ17Arf1-GDP, which is consistent with their comparative thermodynamic and hydrodynamic analyses. Taken together, these experiments suggest an unfolding model for the nucleotide switch of Arf6 and shed new light on its biochemical differences with Arf1.

Introduction

Small GTP-binding proteins of the Arf family are essential to most aspects of intracellular traffic (reviewed by D'Souza-Schorey and Chavrier[1]). Arf1 and Arf6 are the most studied of the five human Arf isoforms. Despite their high sequence homology (>60% identity), Arf1 and Arf6 have nonoverlapping functions in cells (reviewed by D'Souza-Schorey and Chavrier,[1] Gillingham and Munro,[2] and Donaldson and Honda[3]). Arf1 acts mostly at the Golgi, where it promotes the

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Abbreviations used: ArfGEF, Arf-specific guanine nucleotide exchange factor; BFA, brefeldin A; GEF, guanine nucleotide exchange factor; Arf6-GDP^{FL}, full-length Arf6-GDP; HSQC, heteronuclear single quantum coherence; SAXS, small-angle X-ray scattering; SEC-MALS, size-exclusion chromatography–multiangle light scattering; 3D, three-dimensional; EDTA, ethylenediaminetetraacetic acid; PDB, Protein Data Bank.

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recruitment of vesicular coats, while Arf6 functions at the plasma membrane, at the crossroads between trafficking and cytoskeleton processes. The separated localizations of Arf1 and Arf6 were thought for some time to account for their distinct functions in cells. However, recent studies revealed a previously overlooked promiscuity of Arf1 and Arf6 at the plasma membrane, where they may act in a signalling cascade in which both proteins require to be discriminated upon structural criteria.[4,5] In support of the hypothesis that Arf1 and Arf6 differ in their structural properties, *in vitro* studies have shown that Arf-specific guanine nucleotide exchange factors (ArfGEFs) of the EFA6 (exchange *f*actor for *Arf6*) group have a strict specificity for Arf6,[6] and that while Arf1 is sensitive to the fungal drug brefeldin A (BFA), Arf6 is resistant to the drug.[7] Yet, the structures of Arf1-GDP[8] and Arf6-GDP[9] are highly similar, and those of Arf1-GTP[10] and Arf6-GTP[11] are almost indistinguishable. Furthermore, all residues in Arf1 that contact the ArfGEFs or BFA in the crystal structures of Arf1/ArfGEF complexes[10,12,13] are identical between Arf1 and Arf6. No straightforward physicochemical or structural model has been put forward to account for these exquisite specificities.

Dynamics is a more elusive aspect of protein structural "personalities," whose functional importance is increasingly being recognized (reviewed by Henzler-Wildman and Kern[14]). Structural dynamics is central to the conversion of Arf proteins from their inactive GDP-bound conformation to their active GTP-bound conformation. Their GDP/GTP switch involves a considerable conformational change, which has been proposed to ensure the allosteric propagation of information between their membrane-facing side and their nucleotide-binding site (reviewed by Pasqualato *et al.*[15]). A consistent structural model for Arf activation was established from biochemical and crystallographic analyses of spontaneous and guanine nucleotide exchange factor (GEF)-stimulated nucleotide exchange.[8–11,13,15–17] In this model, the myristoylated N-terminal α-helix locks strands β2–β3, which are part of the central β-sheet (the interswitch), in a retracted conformation in Arf-GDP, thereby maintaining Arf-GDP in a cytosolic form that cannot be activated by ArfGEFs. The first event of Arf activation is the binding of Arf-GDP to membranes by its N-terminal α-helix, which requires that the helix be displaced from the GTPase core. This movement unlocks the interswitch, thereby turning membrane-attached Arf-GDP into a substrate for ArfGEFs. ArfGEFs then promote a two-residue register shift of the interswitch, which recovers a conformation that can bind GTP, eventually allowing the replacement of GDP by GTP.

In this work, we sought out structural differences between Arf1 and Arf6 by analyzing the structural and spectroscopic properties of an Arf6 mutant that lacks the N-terminal α-helix, and by comparing them to those of the equivalent Arf1 truncation mutant. The truncated Arf1 mutant has been instrumental in deciphering the mechanism of the nucleotide switch (reviewed by Pasqualato *et al.*[18]). As the truncation of the helix mimics its displacement by membranes, nucleotide exchange can be monitored in solution with exchange kinetics close to those of myristoylated full-length Arf1 in the presence of membranes. It therefore provides a tractable model to study the transient steps of the Arf nucleotide switch that take place as Arf-GDP becomes membrane associated prior to its activation by ArfGEFs. Our analysis reveals the existence of an unexpected structural intermediate that is unique to Arf6 and may account for its unresolved functional specificities.

Results

Δ13Arf6-GDP is partially unfolded in the crystal

We solved the crystal structure of human Δ13Arf6-GDP at 1.8 Å resolution (Table 1). The asymmetric unit comprises two Arf6 subunits related by a pseudo-2-fold axis. Both subunits feature an unexpected rearrangement compared to full-length Arf6-GDP (Arf6-GDP^{FL})[9] (Fig. 1; representative electron density shown in Fig. S1 in Supplementary Data). Residues affected by structural changes extend from Lys34 in strand β1′, which corresponds to switch 1 in Arf-GDP structures, to Asn56 after strand β2 (interswitch) in the central β-sheet. The β1′–β2 region is essentially disordered in one Δ13Arf6-GDP subunit of the

Table 1. Crystallographic statistics

	Arf6-GDP
Data collection	
Space group	$P2_12_12_1$
Unit cell parameters *a*, *b*, *c* (Å)	38.47, 49.72, 143.07
Wavelength (Å)	0.98
Resolution range (inner shell) (Å)	41.0–1.82 (1.93–1.82)
Number of unique reflections	25,041 (3966)
Redundancy	5.3 (5.2)
Completeness (%)	97.7 (97.6)
R_{merge} (%)	7.0 (58.7)
$I/\sigma(I)$	14.5 (2.7)
Refinement statistics	
Resolution range (Å)	40.83–1.82
R/R_{free} (%)	17.05/19.54
Protein atoms	2500
Water molecules	172
Ligand	GDP
RMSD	
Bond length (Å)	0.010
Bond angle (°)	1.08