Crystal structure of Rab9 complexed to GDP reveals a dimer with an active conformation of switch II

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Rab9 is a small Ras-like GTPase, that is predominantly localized at the endosomal membrane. Being a molecular switch, it cycles between an inactive, GDP-bound, and an active, GTP-bound state. Rab9 is involved in recycling of mannose-6-phosphate receptors (MPRs) from endosomes back to the trans Golgi network [1]. The role of Rab9 is supposedly to recruit the adaptor protein TIP47, which binds to the cytoplasmic tails of MPRs [2]. We crystallized Rab9 in the presence of Sr$^{2+}$, which opened the possibility to determine this structure by MAD. A MAD dataset was collected at the Sr$^{2+}$ edge to 2.5Å resolution at DESY beamline BW7A. However, due to the presence of merohedral twinning, MAD phasing using these data was unsuccessful [3]. We therefore determined the Rab9 structure from a slightly twinned 1.77Å data set by molecular replacement using the yeast Rab7 homologue Ypt7p as a search model. Refinement was done with CNS and SHELXL resulting in a final R$_{cryst}$ of 0.16 and R$_{free}$ of 0.22. The crystal structure of Rab9 revealed two molecules in the asymmetric unit, in one of which the nucleotide is coordinated by a Sr$^{2+}$-ion instead of the usual Mg$^{2+}$. Both molecules form a unique dimer via an intermolecular $\beta$-sheet that buries the switch I regions. Surface area and shape complementarity calculations suggest that Rab9 dimers can form an inactive, membrane-bound pool of Rab9-GDP that is independent of GDI. Mg$^{2+}$-bound Rab9 represents an inactive state, but Sr$^{2+}$-bound Rab9-GDP displays activated switch region conformations, mimicking those of the GTP state [4].

Figure 1

Figure 1: The Rab9-GDP asymmetric unit is shown as a ribbon diagram in a projection along the crystallographic fourfold axis. Molecule A (red helices, green sheets), Molecule B (blue helices, purple sheets). The nucleotides are drawn as ball-and-stick models and benzoate moieties as cpk-models.
Figure 2: Representation of the electron density quality of the nucleotides from the two molecules in the Rab9 asymmetric unit. (a) GDP is canonically coordinated by an Mg\(^{2+}\)-ion. (b) GDP is coordinated by a Sr\(^{2+}\)-ion, which makes a total of seven interactions, in which also a benzoic acid moiety is involved.

References