UNUSUAL ARMADILLO FOLD IN THE HUMAN GENERAL VESICULAR TRANSPORT FACTOR P115

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The golgin family gives identity and structure to the Golgi apparatus and is part of a complex protein network at the Golgi membrane. The golgin p115 is targeted by the GTPase Rab1α, contains a large globular head region and a long region of coiled-coil which forms an extended rod-like structure. p115 serves as vesicle tethering factor and plays an important role at different steps of vesicular transport. Here we present the 2.2 Å-resolution X-ray structure of the globular head region of p115. The structure exhibits an armadillo fold that is decorated by elongated loops and carries a C-terminal non-canonical repeat. This terminal repeat folds into the armadillo superhelical groove and allows homodimeric association with important implications for p115 mediated multiple protein interactions and tethering.

Membrane trafficking in eukaryotic cells is an example for the modular organization of cellular activity. The formation and delivery of transport intermediates to specific cellular locations are complex processes that can be divided into several stages [1]. In this modular organization the first interaction of a vesicle and its target membrane is termed tethering. It depends on a heterogeneous group of proteins called ‘tethers’ [2] They can be divided into multi-subunit tethering complexes and proteins containing an extended coiled-coil region.

The golgin p115, which forms stable homodimers, is recruited to membranes in a nucleotide-dependent manner by the guanosine triphosphatase (GTPase) Rab1α [2, 3] and belongs to the family of tethers containing an extended coiled-coil region. p115 is among the best characterized representatives of long coiled-coil tethers. The architecture of p115 comprises a long central coiled-coil region, a large globular N-terminal domain and a C-terminal acidic region. The central region mediates homodimerization and contains the Rab1α binding site. Interaction of Rab1α and p115 is thought to tether coat-protein complex II (COP II) vesicles to each other, thus promoting homotypic vesicle fusion [3]. The C-terminal region of p115 binds to GM130 and giantin, two further coiled-coil tethers localized at the Golgi membrane [4].


To understand how these different activities are combined in one p115 molecule, we embarked on its structure analysis. We used a construct comprising the globular head region of p115 (p115GHH), residues Asp54 to Tyr629) for crystallization. The fragment lacks 53 N-terminal residues that are predicted to be disordered and the C-terminal coiled-coil domain (p115CC).

STRUCTURES OF HUMAN AND MOUSE apoM – SERENDIPITOUS FATTY ACID IN THE BINDING POCKET AND REFOLDING GONE WRONG!

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ApoM is a 25 kDa HDL-associated apolipoprotein and a member of the lipocalin family of proteins. Mature apoM retains its signal peptide, which serves as a lipid anchor attaching apoM to the lipoproteins, thereby keeping it in the circulation [1, 2]. Studies in mice have suggested apoM to be antiatherogenic [3], but its physiological function is yet unknown. We have determined the 1.95 Å resolution crystal structure of recombinant human apoM expressed in E. coli using MAD phasing and made the unexpected discovery that apoM, although refolded from inclusion bodies, was in complex with either myristic acid or glycerol-1-myristate. ApoM displays the typical lipocalin fold characterised by an 8-stranded antiparallel β-barrel that encloses an internal ligand-binding pocket. Fatty acid and lipid binding studies together with competition experiments on apoM and the mutants apoM⁰⁴⁷F and apoM⁰¹⁰⁰F suggested myristic acid and sphingosine-1-phosphate as possible ligands.

We also determined the crystal structure of mouse apoM to a resolution of 2.5 Å. Although both human and mouse homologues of apoM are expected to have the same three-dimensional structures, the structure of mouse apoM revealed a different topology when compared to its human homologue (Figure 1) probably due to mis-refolding.


Figure 1. Topology plot of (a) human apoM and (b) mouse apoM. In mouse apoM the β-strand E is missing and the β-strand F is replaced by the N-terminal long loop.

COOT

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Coot is a program for fitting macromolecular structures to x-ray data.

The majority of new algorithms added to Coot in the last 2 years have been introduced to address the problems of modelling at lower resolutions. At these resolutions, the maps can become misleading, therefore one has to rely on prior knowledge of protein structure for successful model-building.

This presentation shall include discussion of Coot’s low resolution model-building tools, secondary structure restraints, Ramachandran restraints, rotamers and backrubs.

Also, I will include some handy hints for more expeditious usage of Coot.