In vitro protein ligation and its application in structural analysis of lipidated proteins

Alexey Rak, Olena Pylypenko, Thomas Durek, Anja Watzke, Susanna Kushnir, Lucas Brunsveld, Herbert Waldmann, Roger S. Goody, Kirill Alexandrov

ABSTRACT

Rab/Ypt GTPases represent a family of key regulators of membrane traffic in eukaryotic cells. Association of Rab proteins with their targeted membranes is facilitated by posttranslational modifications with isoprenoid lipids. GDP dissociation inhibitor (GDI) is a general and essential regulator of Rab recycling. Although knowledge of the structure of the Rab:GDI complex is central for understanding vesicular transport, progress in its determination has been hampered by the lack of methods for engineering post-translationally-modified proteins. Here we have used a combination of total-chemical synthesis, protein engineering and intein mediated *in vitro* protein ligation to generate preparative amounts of prenylated Ypt1:GDI complex. The structure of the complex was solved to 1.5 Å resolution and provides a mechanistic explanation for the ability of GDI to selectively interact with GDP bound Rab proteins and to inhibit the release of nucleotide. Unexpectedly, we found that the isoprenoid binding site of GDI is formed by the hydrophobic core its domain II. Moreover, the presented structure demonstrates that the I92P mutation of α-GDI, which causes mental retardation in humans, perturbs the fixation of the Ypt/Rab C-terminus on domain I of GDI.