Architecture of heterodimeric phosphoinositide-3-kinase gamma

Xuxiao Zhang and Roger Williams
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, United Kingdom
xuxiao@mrc-lmb.cam.ac.uk

Phosphoinositide-3-kinase gamma (PI3Kγ) is a dual specificity lipid and protein kinase that plays essential roles in innate immune responses. It consists of a p110γ catalytic subunit with a p84 or p101 regulatory subunit. I have taken a crystallographic approach studying the p110/p84 subunit interaction and regulatory mechanism. By employing a range of deletion variants of both subunits, I have obtained crystals of the p110γ/p84 heterodimer that diffract to about 6 Å resolution. Although my current efforts focus on improving the resolution of the crystals, my low-resolution datasets have yielded partially interpretable electron density for the heterodimer and shed some light on how the catalytic and regulatory subunits interact. My results indicate that both N- and C-terminal regions of p84 are necessary for stable formation of the PI3Kγ complex, and they show that the N-terminal Adaptor Binding Domain (ABD) of the p110γ subunit is necessary but not sufficient for high affinity binding to p84. The structure suggests at least one class of additional weaker interactions.

Introduction

- PI3Kγ is a key regulator of inflammatory responses and cardiovascular homeostasis.
- It is activated by heterotrimeric G-protein-coupled receptors.
- Its enzymatic products are important lipid second messengers in a variety of signalling pathways.
- The structure of the p110 catalytic subunit has been partially solved.
- Regulatory subunit, p101 or p84, enables translocation and substrate specificity of p110.
- Both the structure of regulatory subunit and the mechanism by which it regulates the enzyme remain unknown.

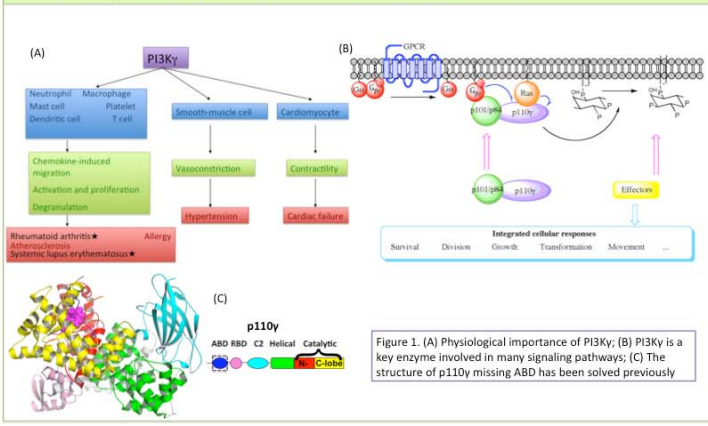


Figure 1. (A) Physiological importance of PI3Kγ; (B) PI3Kγ is a key enzyme involved in many signaling pathways; (C) The structure of p110γ missing ABD has been solved previously

- Crystallization of p110γ/p84 produced crystals that diffracted to 6 Å and datasets at 8 Å resolution were collected.
- Electron density map shows 4 molecules of p110γ per asymmetric unit.
- Unassigned density is found between C2 and catalytic domain of p110γ, possibly analogous structural arrangement with iSH2 of p85 and p110α. (Note: p85 is completely unrelated in sequence to p84 or p101).

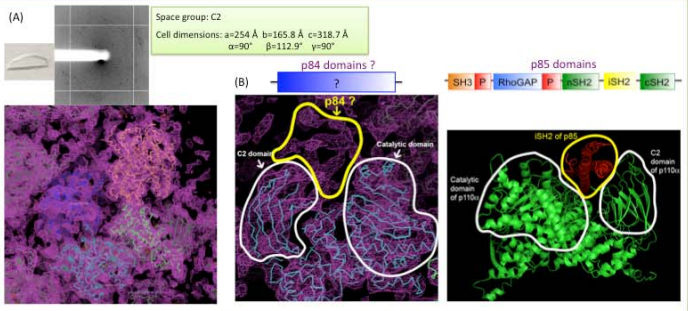
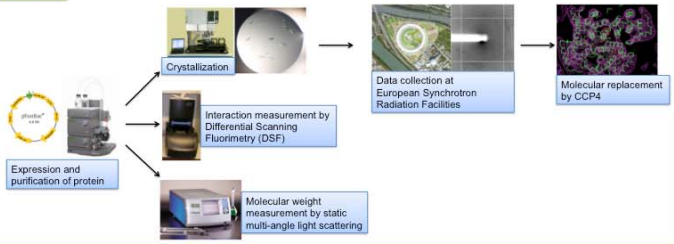


Figure 5. (A) A diffraction image of a p110γ/p84 crystal with its 8 Å resolution electron density map from molecular replacement; (B) Unassigned electron density found between C2 and the catalytic domain of p110γ (left) and analogous structure in PI3Kα (right).

Methods



Results

- Expression and purification of the p110γ/p84 complex produces a high yield of stable protein in Sf9 cells.
- Multi-angle light scattering indicates 1:1 ratio of p110γ and p84 in the complex.

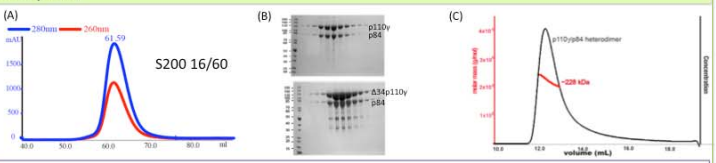


Figure 2 (A) Gel filtration profile of p110γ/p84 complexes; (B) Gel images of purified protein complexes; (C) Molecular weight of p110γ/p84 complex determined by SEC-MALS. The chromatogram recorded by the differential refractometer and the Mw across the peak are shown.

- Deletion mutants Δ34p110γ and Δ144p110γ were co-expressed with p84.
- Δ144p110γ interacts with p84 weakly as small amount of p84 was pulled down by His_6-tagged Δ144p110γ when incubated with Ni-NTA beads.
- DSF indicates single phase curve for full length p110γ or Δ34p110γ mixture with p84, but biphasic curve for Δ144p110γ mixture with p84.

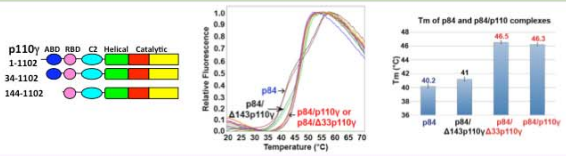


Figure 3. DSF melting curves and comparison of first phase Tm of p84 and its stoichiometric mixture with p110γ deletion mutants.

- Deletion mutants of p84 were all insoluble.
- Western blot identified that the 2 major proteolytic products at around 50kDa and 35kDa in full-length p110γ/p84 complex contain truncated N-terminus of p84.
- Mass spectrometry fingerprinting indicates p84 was probably proteolyzed into heterogeneous fragments that still bind p110γ.

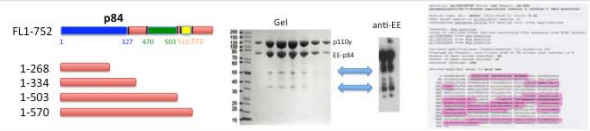


Figure 4 Gel, western blot profiles and mass spectrometry fingerprinting results of the proteolytic products of p110γ/p84.

Conclusions

- ✧N-terminal region (residues 34-143) of p110γ is necessary for high affinity binding to p84
- ✧Some weaker interactions also exist between the rest of p110γ (residues144-1102) and p84
- ✧Both N- and C-terminal regions of p84 are necessary for stable formation of PI3Kγ complex
- ✧Low-resolution X-ray datasets yielded molecular replacement solutions that appear to have interpretable density
- ✧Future: (1) Improve the resolution (2) Interpret the electron density map by heavy metal phasing