Studying the Molecular Mechanism of Peptidyl Prolyl Isomerase Pin1

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Introduction

About a decade ago a combination of post-translational modification and peptidyl-prolyl isomerization has been revealed as one of Nature's strategies to fine-tune regulatory pathways involved in cell cycle control, cell proliferation and apoptosis [1]. One of the crucial players in these systems is the enzyme Pin1, a peptidyl-prolyl isomerase, which specifically recognizes phosphorylated (p)Ser/Thr-Pro modules [2]. What followed was an overwhelming amount of published data describing Pin1's contribution in phosphorylation-mediated signaling and its many targets implicated in a large number of devastating human diseases [3,4]. Despite that the molecular mechanism of how this enzyme controls the diversity of signaling events remains elusive due to missing quantitative biophysical approaches.

Experimental

In order to study how the two domains of Pin1 (WW and catalytic domain) interact during catalysis thermodynamic and kinetic data were extracted from NMR 2D ^{15}N HSQC titrations and 2D ^{15}N ZZ exchange experiments [5,6]. The latter solution studies were carried out at the National High Magnetic Field Laboratory in Tallahassee (FL) using the Varian 600MHz and Varian 720MHz instruments. Various temperatures, protein concentrations and different substrates of Pin1 have been explored. Additional 3D experiments were carried out for the calculation of the enzyme-substrate-complex.

Results and Discussion

The human metabotropic glutamate receptor type 5 (mGluR) is a G-protein coupled membrane protein and has been shown to be an important Pin1 substrate involved in synaptic plasticity and memory function [7]. Binding studies carried out by isothermal calorimetry (ITC) and NMR indicate a cooperative binding mechanism for both Pin1 domains towards double phosphorylated mGluR substrate. The Pin1 binding motif in mGluR is located within the intrinsically disordered C-terminal segment (ALpTPPpSPFR). Interestingly, while Pin1 binds with moderate-to-high affinity towards mGluR at equimolar concentrations (K_D=2-3µM), we find an additional binding mode highly dependent on substrate concentration. The latter is likely due to the molecular binding mode switching from 'intra' to 'inter' meaning that two mGluR molecules saturate one Pin1 molecule. Even more fascinating, the 'intra' (1:1) binding mode shows at least three distinct, but equally populated mGluR species bound to Pin1. Structure calculations of one of those species confirm the cooperative binding mechanism at the 1:1 ratio. Further studies will focus on the remaining mGluR species and their relevance in biology.

Conclusions

Single phosphorylation sites in Pin1 substrates do not present the full picture of events happening during turn-over. Most likely other phosphorylation sites further away are important players for the molecular mechanism by which Pin1 recognizes and efficiently catalysis *cis*-to-*trans* isomerization of pSer/pThr-P modules.

Acknowledgements

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References

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