MS11-P07

Crystal structure of a mammalian pseudokinase reveals an original dimerization

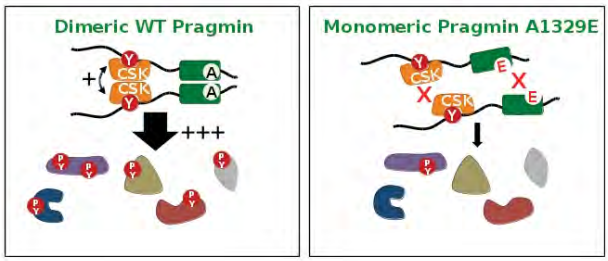
Gilles Labesse[1] Céline Lecointre[2], Valérie Simon[2], Clément Kerneur[2], Frédéric Allemand [1], Ingrid Montarras[2], Muriel Gelin[1], Serge Urbach[3], Serge Roche[2]

1. Centre de Biochimie Structurale, CNRS, Montpellier, France
2. Centre de Recherche de Biologie cellulaire de Montpellier, CNRS, Montpellier, France
3. Institut de Génomique Fonctionnelle, CNRS, Montpellier, France

email: labesse@cbs.cnrs.fr

Although partially or totally devoid of any ATP binding or hydrolysis, pseudokinases are now recognized as key players in cell signaling. However, their functioning is still unclear for a number of such pseudokinases encoded in the mammalian genomes. Here we describe the crystal structure of the folded region of SGK223, a large pseudokinase from rat. We could solve the crystal structure by molecular replacement, at a 3.0 A resolution, despite a low overall sequence identity (20-25% over the whole kinase domain), using the software Phenix, an ensemble of partial models built using our server @TOME-2 and data recorded automatically on the beamline MASSIF-1 at the ESRF synchrotron.

The structure contains a classical protein kinase fold, devoid of any ATP-binding activity. It also highlighted several sequence motifs conserved in other psudokinases and extend the corresponding superfamily. Interestingly, these pseudokinases possess N- and C-terminal extensions forming an original dimerization domain. This dimeric pseudokinases have been linked to cancer by up-regulating protein tyrosine phosphorylation. Our results suggest a structural model for understanding how pseudokinases induce protein tyrosine phosphorylation [1,2].



References:

[1] Dimerization of the Pragmin Pseudo-Kinase Regulates Protein Tyrosine Phosphorylation. Lecointre C, Simon V, Kerneur C, Allemand F, Fournet A, Montarras I, Pons JL, Gelin M, Brignatz C, Urbach S, Labesse G, Roche S. Structure. 2018, 26, 545-554.
[2] A Pseudo-Kinase Double Act. Preuß F, Mathea S, Knapp S. Structure. 2018, 26, 527-528 (preview)

Keywords: cell signaling, cancer, protein-kinase

MS11-P08

Structure and function of DJ-1 superfamily proteins from staphylococcus aureus

Ae-Ran Kwon[1], Hyo Jung Kim[2]

1. College of Herbal Bio-Industry, Daegu Haany University, Gyeongsan-City, Korea South
2. School of Pharmacy, University of Nottingham, Nottingham, United Kingdom

email: arkwon@dhu.ac.kr

The DJ-1/ThiJ/PfpI superfamily of proteins is highly conserved across all biological kingdoms showing divergent multifunctions, such as chaperone, catalase, protease, and kinase. The common theme of these functions is responding to and managing various cellular stresses. Most members of the DJ-1/ThiJ/Pf pI superfamily are oligomers and are classified into subfamilies depending on discriminating quaternary structures (DJ-1, YhbO and Hsp types).

SAV1875, a conserved protein from Staphylococcus aureus, is a member of the YhbO-type subfamily. The crystal structure of SAV1875 from S. aureus was determined. The cysteine residue located in the dimeric interface (Cys105) forms a catalytic triad with His106 and Asp77, and it is spontaneously oxidized to Cys105-SO_2H in the crystal structure. To study the oxidative propensity of Cys105 and the corresponding functional differences with changes in cysteine oxidation state, the crystal structures of SAV1875 variants E17N, E17D and C105D, and over-oxidized SAV1875 were determined. We identified SAV1875 as a novel member of the YhbO-type subfamily exhibiting chaperone function. However, if SAV1875 is over-oxidized further with H_2O_2, its chaperone activity is eliminated. On the basis of our study, we suggest that SAV1875 functions as a chaperone and the redox state of Cys105 may play an important role.

The Hsp-type subfamily includes Hsp31, a chaperone and glyoxalase III. SAV0551, an Hsp-type subfamily member from Staphylococcus aureus, is a hypothetical protein that is predicted as Hsp31. Thus, to reveal the function and reaction mechanism of SAV0551, the crystal structure of SAV0551 was determined. We have shown that SAV0551 functions as a chaperone and that the surface structure is crucial for holding unfolded substrates. As many DJ-1/ThiJ/PfpI superfamily proteins have been characterized as glyoxalase III, our study also demonstrates SAV0551 as a glyoxalase III that is independent of any cofactors. We have confirmed that the components required for reaction are present in the structure, including a catalytic triad for a catalytic action, His78 as a base, and a water molecule for hydrolysis. Our functional studies based on the crystal structures of native and glyoxylate-bound SAV0551 will provide a better understanding of the reaction mechanism of a chaperone and glyoxalase III.

References:

1. Kim HJ, Kwon AR, & Lee BJ. (2016) Biochem. J. 473(1), 55-66.
2. Kim HJ, Lee KY, Kwon AR, & Lee BJ. (2017) Biosc. Rep. 37(6), BSR20171106

Keywords: DJ-1/ThiJ/PfpI superfamily, Staphylococcus aureus