Probing the Structural and Biochemical Basis for the Development of Cushing's Syndrome Caused by a Somatic Mutant of Protein Kinase A

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Protein phosphorylation is a ubiquitously conserved signaling mechanism playing a crucial role in activating or deactivating vital cellular signaling pathways. Dysregulation of these pathways lead to human diseases, such as heart disease and cancer. Recently somatic mutations in the gene *PRKACA* coding for the catalytic domain of cAMP-dependent protein kinase A (PKA-C) have been identified as frequent genetic alterations in adrenocortical adenomas responsible for adrenal Cushing's syndrome. One such mutation, L205R is located at the interface between the catalytic and regulatory subunits of PKA. Crystal structures of L205R complexed with ATP and the short-length of the heat stable protein kinase A inhibitor (PKI_{5-24}) are mostly superimposable to the wild-type PKA-C structure, with the exception of the orientation of R205 side chain, that is reflected in a slightly different positioning of the pseudo-substrate.

To understand how this mutation affects both the regulation and activity of PKA-C, we performed thermostability, kinetics, and binding analyses using biochemical and biophysical techniques as well as NMR spectroscopy. We discovered that the mutation causes a significant reduction in binding affinity for substrates and regulatory domains. In addition, preliminary NMR dynamic studies show that PKA-C L205R exhibits increased conformational dynamics, suggesting that this single-site mutation might influence the allosteric communication within the enzyme. Based on these results, we propose that this somatic mutation might alter the binding interface as well as the conformational dynamics of the enzyme causing aberrant phosphorylation and contributing to the development of the disease.