ABSTRACT
Abstract

The evolutionarily conserved enzymes of glycolytic pathway acting as target for growth signals or oncogenic induction induce shift in cellular metabolism on requirement, providing a reason for metabolism of cancer cells to differ from normally dividing and resting cells. Understanding these processes precisely has allowed Pyruvate Kinase M2 (PKM2), a glycolytic enzyme, to emerge as a major "metabolic modulator". The enzyme involved at the last step of glycolysis, is essential for cell division. Its down-regulated activity allows accumulation of glycolytic intermediates required for biosynthesis of DNA, lipids and amino acids during cell division. An analysis of any aberration of PKM2 (not known, except for one report) in a naturally occurring pathological condition becomes important to study to determine its role as a "metabolic modulator" which has remained enigmatic till date. The present work involved the study of impact of two missense mutations in PKM2 (H391Y and K422R) which were detected in a Bloom syndrome (BS) patient and a cell line; the syndrome known to suffer from multiple phenotypes including cancer at an early age with unknown reasons.

The mechanism of regulation of the activity and the allosteric behavior of pyruvate kinase M2 (PKM2) enzyme and two of its missense mutations was studied by kinetics experiments. The results showed that despite mutations at Inter-subunit contact domain, the mutant proteins maintained homotetrameric structure similar to wild type (as assessed in gel filtration chromatography); but, with a loss of activity by 75% and 20% in K422R and H391Y mutants, respectively. Interestingly, H391Y mutant showed a 6 fold increase in affinity toward its substrate, PEP, and behaved like a non-allosteric protein with compromised cooperative binding, predicted in isothermal titration calorimetry too; whereas, the affinity towards PEP was lost significantly in K422R mutant. H391Y mutant also showed enhanced thermal stability in terms of activity and secondary structure unfolding as predicted by kinetics, circular dichroism spectroscopy and differential scanning calorimetry experiments. The same mutant showed stability (in terms of activity) over a range of pH, lesser effect of the allosteric inhibitor phenylalanine (Phe) and resistance toward structural alteration upon binding of the activator (FBP) and inhibitor (Phe). Both the mutants showed a slight shift in optimum pH from 7.4 to 7.0 for their activity. It was hypothesized how a multifunctional protein could modulate its structure and regulate its function to probably adapt to stressful microenvironment (pH and temperature fluctuations) to favor tumor promotion in cancers.
In order to understand the structural functional correlation, *in vitro* experiments were designed. As the observed mutations were heterozygous in nature, where each cell would express normal as well as mutant monomers of PKM2 (under a bi-allelic expression condition), it was observed experimentally that the mutant protein showed a physical (monomer-monomer) interaction and co-localization with wild (non-mutant) PKM2, behaving in a dominant negative fashion. The cross monomer interaction significantly altered the oligomeric status of homo-tetrameric PKM2 by favoring dimerisation and hetero-tetramerisation, confirmed by cross-linking and density gradient experiments. *In silico* study provided an added support to show that hetero-oligomerisation was energetically favorable. The hetero-oligomeric population of PKM2 showed modulated activity and affinity. The phenotypic implication of such oligomeric structures of PKM2 in a cell in *in vitro* experimental conditions showed that the co-expression of wild and mutant PKM2 result in an increased growth rate of *E. coli* as well as mammalian cells, along with an increased rate of polyploidy. The feature of down-regulation of PKM2 activity by dimerisation is known to be essential to tumor progression. The present study, however, provides a scope beyond the conventional thinking of PKM2 dimerisation and its role in tumor progression since the less active hetero-tetramers in the study also showed their association with increased growth rate and polyploidy, indicating their potential role in favoring cancer progression. The study provides an insight in understanding the modulated role of large oligomeric multifunctional proteins, such as PKM2 to affect cellular behavior, an essential observation to: i) understand tumor sustenance and progression, especially in Bloom syndrome background and ii) design therapeutic intervention in future.