Biochemical characterization of glycerol-3-phosphocholine as a novel PKM2 allosteric regulator

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Abstract

M2-pyruvate kinase (PKM2) which catalyzes the last-step reaction in glycolysis by converting phosphoenolpyruvate (PEP) into pyruvate plays an important role in glucose metabolism. PKM2 (exon 10) or PKM1 (exon 9) are exclusively alternative spliced isoforms from *PKM.* PKM2 exists as a tetramer, dimer or monomer whereas PKM1 is constitutively tetrameric. Furthermore, the activity and oligomeric status of PKM2 can be allosterically regulated by various small molecules such as fructose-1,6-bisphosphate (FBP) and phenylalanine. Previously, PKM2 was reported to activate lipogenesis in hepatocellular carcinoma, indicating a regulatory role in lipid metabolism. To characterize whether PKM2 is allosterically regulated by lipid metabolites, we screened a number of small lipid molecules by using lactate dehydrogenase (LDH)-coupled pyruvate kinase activity assay. Of them, glycero-3-phosphocholine (GPC) potently downregulated the activity of PKM2. The coupling effect was demonstrated by the plot of K_m(PEP) as a function of GPC concentration. On the other hand, GPC hardly affected PKM1's activity, suggesting that GPC is a PKM2-specific effector. Size-exclusion chromatographic profiling revealed that the addition of GPC shifts the equilibrim from tetramer to dimer and/or monomer via a dose-dependent manner. These results together suggest that GPC, a metabolite of choline metabolism, functions as a new allosteric regulator of PKM2. Further investigation of its role in carcinogenesis can provide insights for developing theraputic strategies.

Key words: PKM2, GPC, allosteric regulator.