Scaffolding Function of PI3Kgamma Emerges from Enzyme's Shadow

Maradumane L Mohan and Sathyamangla V Naga Prasad

Department of Molecular Cardiology, *Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA*

Correspondence to Maradumane L Mohan and Sathyamangla V Naga Prasad:*. NB50, Department of Molecular Cardiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA. mohanm@ccf.org; prasads2@ccf.org*
http://dx.doi.org/10.1016/j.jmb.2017.01.023
Edited by Patrick Griffin

Abstract

Traditionally, an enzyme is a protein that mediates biochemical action by binding to the substrate and by catalyzing the reaction that translates external cues into biological responses. Sequential dissemination of information from one enzyme to another facilitates signal transduction in biological systems providing for feed-forward and feed-back mechanisms. Given this viewpoint, an enzyme without its catalytic activity is generally considered to be an inert organizational protein without catalytic function and has classically been termed as pseudo-enzymes. However, pseudo-enzymes still have biological function albeit non-enzymatic like serving as a chaperone protein or an interactive platform between proteins. In this regard, majority of the studies have focused solely on the catalytic role of enzymes in biological function, overlooking the potentially critical non-enzymatic roles. Increasing evidence from recent studies implicate that the scaffolding function of enzymes could be as important in signal transduction as its catalytic activity, which is an antithesis to the definition of enzymes. Recognition of non-enzymatic functions could be critical, as these unappreciated roles may hold clues to the ineffectiveness of kinase inhibitors in pathology, which is characteristically associated with increased enzyme expression. Using an established enzyme phosphoinositide 3-kinase γ, we discuss the insights obtained from the scaffolding function and how this non-canonical role could contribute to/alter the outcomes in pathology like cancer and heart failure. Also, we hope that with this review, we provide a forum and a starting point to discuss the idea that catalytic function alone may not account for all the actions observed with increased expression of the enzyme.

Introduction

Proteins are the building blocks of all living organisms that can be broadly categorized into two groups: structural proteins and biologically active proteins. Structural proteins are the key main constituents of our bodies that contribute to the shape and elasticity of a cell, organ, or body. Biologically active proteins can be subdivided into contractile, transport, regulatory, storage, protective, or enzymes. Enzymes catalyze biochemical reactions in the cells wherein a complex sum of their activity accounts for the response of cells/organs. Enzymes can be further classified into several groups based on the biochemical reactions they perform. The importance of the enzymatic function is reflected by the fact the even the simplest of organisms have hundreds of enzymes in a living cell catalyzing key reactions that are critical for life. Current classification system is designed based on the general class of reaction the enzymes catalyze. The most common broad umbrella of enzyme classes can be categorized into oxidoreductases, ligases, hydrolases, lyases, isomerases, and transferases.

The enzyme of interest for the present review and discussion belongs to the class of transferases. They are the most comprehensively studied enzyme class in biological systems as they are involved in the acute regulation of signaling mechanisms manifesting in responses to external cues. Transferases are enzymes that catalyze the movement/transfer of a functional group/moiety from one molecule to another. In this process, they mediate the activation/inhibition of the

proteins that receive the functional group undergoing post-translational modification. These functional groups/moieties are very diverse including the most common studied moieties like phosphate, methyl, glycosyl, or ubiquitin groups.

Among the large repertoire of transferases, kinases have gained significant prominence due to their role in acute signal transduction mechanisms. Importantly, kinase function gained importance due to their integral role in the regulation of glycolytic pathways determining the energy generation. Specifically, kinases are involved in catalyzing the transfer of phosphate group moieties onto molecules, leading to the phosphorylation of the molecules. The effects of these kinases are wide-ranging as they can act on a variety of molecules including lipids, proteins, carbohydrates, and nucleotide. Such an event of phosphorylation is an example of priming a pathway for accelerated movement of information package from the beginning of chain to the end like in the metabolic pathway of glycolysis or the signal transduction pathway of extracellular regulated kinase 1/2 activation by the upstream mitogen-activated protein kinase pathway. The kinases are a very diverse set of enzymes, and there are more than 500 different kinases that have been identified in humans. The most commonly studied and characterized kinases are lipid and protein kinases. In this regard, there has been intense interest and resources that have been invested in determining the underlying mechanisms of phosphorylation by protein kinases, with protein kinase A (PKA) being one of earliest kinases to be studied [1,2].

In contrast to the classical paradigm that enzymes are designed for catalytic kinase functions, studies have identified many proteins that have conserved structural components of enzymes and yet do not harbor catalytic activity. Such observations opened up the idea of “dead enzymes” or “pseudo-enzymes”, which have characteristic feature of enzymes but, in many cases, lack key amino acids involved in mediating catalysis [3]. The synopsis of protein kinases with non-catalytic functions has been elegantly reviewed previously [4]. One of the earliest identified pseudo-enzymes was α-lactalbumin that has significant homology to lysozyme but has no identifiable lysozyme activity [5,6]. The expression of these pseudo-enzymes is intriguing given that these non-catalytic proteins are still retained in the genome despite significant evolutionary pressure [5], suggesting critical biological roles that are non-enzymatic. Consistent with this idea, pseudo-enzymes have been found to assist in the folding of functional enzymes, provide platform for interactions, and/or act as escort for proteins [7,8].

As our understanding evolves with the use of state-of-art technology, so is the case with the classification of these enzymes. The classification of the enzymes into the pseudo-enzyme cohort is based on the current understanding and can change with new discovery or structural information. For example, integrin-linked kinase was originally thought to be a kinase; however, the structural determination of a fragment of integrin-linked kinase showed that it is not a kinase but instead serves a structural role linking the cell's cytoskeleton to surface receptors [9,10]. On the other hand, Ca^{2+}/calmodulin-activated Ser-Thr kinase was initially classified as a pseudo-enzyme. However once the structure for Ca^{2+}/calmodulin-activated Ser-Thr kinase was solved, it became apparent that it could use alternative amino acids in the kinase reaction and can be reclassified as a kinase [11,12]. These observations indicate that the retention of these pseudo-enzymes in the genome could have functions that we currently are unaware of and that increasing technology would allow us to better understand their roles.

Analysis of the enzyme classification based on their function highlights a key issue that has been overlooked in the context of catalytic enzymes, as functional enzymes harbor all structural components that could serve the non-catalytic duties. Thus, the idea that catalytic enzymes can simultaneously, in parallel, execute both kinase and kinase-independent functions was appreciated with the discovery of the scaffolding function for Pbs2P protein in yeast [13]. One of the classes of enzymes that has been gaining significant scientific appreciation on kinase-independent scaffolding functions in addition to its

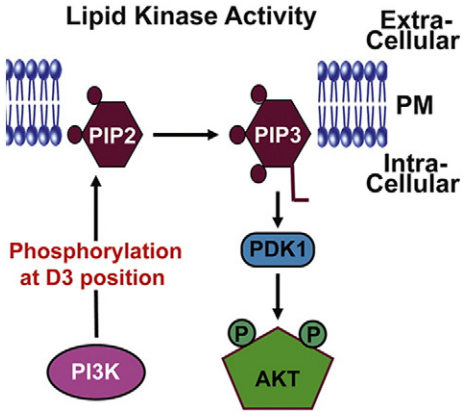

Fig. 1. Lipid kinase activity. PI3Ks are classically well known for this lipid kinase activity. Cytosolic PI3K is recruited to the plasma membrane following an extracellular signal that could range from growth factors like insulin and epidermal growth factor to hormones like epinephrine and norepinephrine, which are G-protein-coupled receptor agonists. Class I PI3Ks will use the phosphoinositide 4,5-bis-phosphate [PtdIns (4,5)P2 or PIP2], a component of the inner leaflet of the plasma membrane, as a substrate to generate D-3 phosphorylated PIP3 products. These D-3 phosphoinositides activate multiple downstream signaling pathways including the well-known anti-apoptotic pathway of phosphoinositide-dependent kinase 1 (PDK1)–protein kinase B (Akt) axis. PI3K, phosphoinsotide 3-kinsae; PM, plasma membrane.