Mol Cell Biochem
DOI 10.1007/s11010-013-1871-8

Fyn kinase regulates type II PtdIns 4-kinases in RBL 2H3 cells

Naveen Bojjireddy · Ranjeet Kumar Sinha · Gosukonda Subrahmanyam

Received: 27 May 2013 / Accepted: 18 October 2013

Abstract Type II phosphatidylinositol 4-kinases are implicated in FcεRI-mediated signaling cascades leading to release of inflammatory molecules. Cross-linking of FcεRI on RBL 2H3 cells results in protein tyrosine phosphorylation and activation of type II PtdIns 4-kinase activity. Protein tyrosine kinase(s) that phosphorylate type II PtdIns 4-kinase(s) in RBL 2H3 cells remains elusive and is being addressed in this manuscript. Anti-Fyn kinase antibodies co-immunoprecipitated type II PtdIns 4-kinase activity from FcεRI cross-linked RBL 2H3 cells. In reciprocal assays, His-tagged types II PtdIns 4-kinases were shown to pull down Fyn kinase. Further, anti-Fyn immunoprecipitates were shown to phosphorylate type II PtdIns 4-kinase α and β in in vitro assays. Pull down studies with GST-Fyn-SH2 and GST-Fyn-SH3 domains showed that type II PtdIns 4-kinases associate with Fyn-SH2 domain. Knockdown of Fyn kinase in RBL 2H3 cells abrogated activation of type II PtdIns 4-kinase activity in response to FcεRI cross-linking and type II PtdIns 4-kinase activity in anti-phosphotyrosine immunoprecipitates. Knockdown of Fyn kinase was also strongly correlated with a reduction in β-hexosaminidase release in response to FcεRI cross-linking. These results suggest that type II PtdIns 4-kinases act downstream of Fyn kinase in FcεRI signaling cascades and are regulated by Fyn kinase.

N. Bojjireddy · R. K. Sinha · G. Subrahmanyam
Department of Biosciences and Bioengineering, Wadhwani Research Centre for Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India

Present Address:
N. Bojjireddy (✉)
Department of Anesthesiology and Pain Medicine, University of Washington, 850 Republican St, Room No: N130, Seattle, WA 98109, USA
e-mail: naveenb@u.washington.edu

Keywords FcεRI receptor · Phosphatidylinositol 4-phosphate · SH2 domain · β-Hexosamindase

Introduction

Cross-linking of high affinity IgE receptors (FcεRI) on mast cell surface leads to activation of various cellular processes leading to degranulation of inflammatory mediators causing type I hypersensitivity reaction [1–6]. FcεRI is a heterotetrameric receptor complex consisting of one α subunit, one β subunit, and two γ subunits interconnected by disulfide linkage [7]. The α subunit binds to IgE, whereas β and γ subunits are involved in signal transduction through their specialized motifs called immunoreceptor tyrosine-based activation motifs (ITAMs) [8, 9]. The early signaling events that occur during the FcεRI-mediated mast cell activation include activation of protein tyrosine kinases, phosphorylation of ITAM motifs of β and γ subunits, hydrolysis of phosphatidylinositol 4,5 bisphosphate (PtdIns4,5P_2) into diacylglycerol and inositol 1,4,5 tris phosphate [10–15].

Mast cells express several protein tyrosine kinases Lyn, Fyn, Syk. Lyn, and Fyn kinases belong to non-receptor src family protein tyrosine kinases and are associated with FcεRI. Lyn kinase phosphorylates ITAMs on FcεRI receptors of β and γ chains. Phosphorylated ITAMs on γ chain provide docking sites for another tyrosine kinase Syk which plays a key role in FcεRI signaling [16]. Even though Fyn kinase does not appear to phosphorylate FcεRI receptors, its activity is required for degranulation and cytokine secretion [17]. Association of these protein tyrosine kinases with FcεRI receptor and a concomitant protein tyrosine phosphorylation and activation of type II PtdIns

Published online: 31 October 2013