Bioorganic & Medicinal Chemistry Letters 24 (2014) 2334–2339

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Small chemicals with inhibitory effects on PtdIns(3,4,5)P_3 binding of Btk PH domain

Youngdae Yoon *

Department of Environmental Health Science, Konkuk University, Seoul 143-701, Republic of Korea

ARTICLE INFO

Article history:
Received 26 December 2013
Revised 10 March 2014
Accepted 22 March 2014
Available online 1 April 2014

Keywords:
Btk PH domain
PtdIns(3,4,5)P_3
In silico docking
Small chemicals

ABSTRACT

Phosphatidylinositol-3,4-5-triphosphates (PtdIns(3,4,5)P_3) formed by phosphoinositide-3-kinase (PI3K) had been known as a signaling molecule that plays important roles in diverse cellular processes such as cell signaling, metabolism, cell differentiation, and apoptosis. PtdIns(3,4,5)P_3 regulates diverse cellular processes by recruiting effector proteins to the specific cellular locations for correct functions. In this study, we reported the inhibitory effect of small chemicals on the interaction between PtdIns(3,4,5)P_3–Btk PH domain. Small chemicals were synthesized based on structural similarity of PtdInsP head-groups, and tested the inhibitory effects in vitro via surface plasmon resonance (SPR). As a result, the chemical **8** showed highest inhibitory effect with 17 μM of IC_{50} value. To elucidate diverse inhibitory effects of different small chemicals we employed in silico docking experiment using molecular modeling and simulation. The result of docking experiments showed chemical **8** has more hydrogen bonding with the residues in PtdIns(3,4,5)P_3 binding site of Btk PH domain than others. Overall, our studies demonstrate the efficient approach to develop lipid binding inhibitors, and further we can use these chemicals to regulate effector proteins. In addition, our study would provide new insight that lipid binding domain may be the attractive therapeutic targets to treat severe human diseases.

Phosphatidylinositol phosphates (PtdInsPs) are one of minor components of cellular membrane, and they play important roles in cellular signaling by recruiting proteins possessing lipid binding domains,[1–3] such as Epsin1 N-terminal homology (ENTH),[4,5] Ap180 N-terminal homology (ANTH),[6] phox homology (PX),[7] and pleckstrin homology (PH) domains,[8] to the specific cellular membrane. PH domains are one of most well characterized PtdInsPs binding protein modules found in proteins involved in many cellular processes, such as cell signaling, proliferation, migration, cell growth, cytoskeletal organization, regulation of intracellular membrane transport and modification of membrane phospholipids.[1,8–10] The majority of PH domain-containing proteins require membrane association for their functions, and PH domain interacts directly with cell membrane by binding to PtdInsPs with a broad range of specificity.[2,11,12] This specific binding of the PH domain to PtdInsPs can be necessary and sufficient for the correct recruitment of the host protein to the membrane.

The most negatively charged phosphoinositide, PtdIns(3,4,5)P_3 regulates various PH domain containing proteins, such as serine-threonine kinases Akt and PDK1, general receptor for phosphatidylinositides protein 1 (GRP1), protein tyrosine kinases of the Bruton's tyrosine kinase (Btk), and a GDP/GTP exchange factor of ADP ribosylating factor 6 (Arf6).[9,13] The generation of PtdIns(3,4,5)P_3 by PI3K in the response to the various cellular stimuli recruits those proteins from cytosol to the specific cellular membrane to function through PH domain mediated interaction.[14,15] Moreover, this type of lipid-lipid mediated cellular signaling is tightly regulated by complex mechanisms of multiple signaling components. Therefore, malfunction in this type of signaling is known to be involved in various severe human diseases.[16,17]

For example, the hyper-activation of the enzymes in lipid-mediated signaling pathway causes several human diseases.[12,18] Therefore, the effector proteins in lipid-mediated cellular signaling had been targeted for new drug developments in pharmaceutical industry.[17,19] Even though it had been known the interaction between PtdInsP and PH domain is essential for activation of enzymes, surprisingly most of cases the functional domains had been the therapeutic targets for drug discovery. However, it was not easy to develop new drugs targeted function domains, because of the complexity of cellular functions and side effects coming from disrupting downstream pathways. Therefore, it is necessary to have new targets to inhibit the activities of these proteins, and lipid binding site of them could be attractive therapeutic targets.

There are several agonists for effector proteins involved in lipid-mediated cellular signaling. For example, LY294002 inhibits

* Tel.: +82 24500443.
E-mail addresses: yyoon21@gmail.com, shim0924@konkuk.ac.kr

http://dx.doi.org/10.1016/j.bmcl.2014.03.068
0960-894X/

PtdIns-3-P kinase (PI3K) resulting PtdIns(4,5)P_2 level decrease,[20] and U73122 inhibits phospholipase C (PLC) activity for reducing inositol triphosphate (IP3).[21] However, all of them were targeted to the functional domain of effector proteins to decrease activities. On the other hand, the efforts to inhibit the interaction between membrane-lipid binding domains are relatively less. Recently, it was reported that protein kinase C (PKC) was inhibited by chemically synthesized hydroxymethyl phenyl ester analogues and alkyl cinnamates by disrupting the interaction between diacylglycerol (DAG)-C1 domain.[22,23] And also the chemical compounds called PITENINs were reported to inhibit PH domains of Akt/PDK1 in PI3K/PDK1/Akt pathway, and the same compound showed inhibition of ARNO/GRP1 PH domains.[24,25] All PH domain mentioned above were known to strongly interact with PtdIns(3,4,5)P_3. Therefore, the PITENINs showed the inhibitory effect on the interaction between PtdIns(3,4,5)P_3 and PH domains, and PITENINs were used to regulate PtdIns(3,4,5)P_3 related cellular signaling pathway such as PI3K/Akt pathway and to suppress cell migration via ARF6 inhibition. In this case, PITENINs were screened from about 50,000 small molecule library using high throughput screening. Because the high throughput screening needs specific equipment and is expensive, it was not available for all research groups. Therefore, it is needed to have new approaches to develop lipid binding inhibitors based on the rational design to improve screening efficiency.

The small chemicals tested in this study were generously provided from Dr. Daesung Lee at University of Illinois at Chicago, and they were designed based on the structural similarity to the head group of PtdInsP and chemically synthesized. Three sets of compound backbones were designed, and each of them has at least one phosphate group. Each set of compound has small variations, such as addition of carboxyl group and phosphate group, and substitution of hydroxyl group with methoxy or ethoxy groups (Fig. 1). The aim of this research is to develop small chemicals as antagonists to down-regulate activity of effector proteins by inhibiting lipid binding activity, and to suggest the lipid-protein interaction would be the potential therapeutic targets in pharmaceutical industry.

For this study, we selected Btk PH domain as a target lipid binding domain which has known to specifically bind to PtdIns(3,4,5)P_3, and this interaction is critical for the activation of Btk.[26] The DNA encoding Btk PH domain (1–156 amino acid) was cloned into pET-21(a) vector and expressed with 6xhistidine tag in *Escherichia coli* BL21(DE3) RIL cells (Invitorgen). The protein expression and purification were same as described previously.[27]

For in vitro assay to determine the inhibitory effect of small chemicals on lipid binding of Btk PH domain, surface plasmon resonance (SPR) were employed. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine (POPS) were purchased from Avanti Polar Lipids, and a 1,2-dipalmitoyl derivative of phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) and phosphatidylinositol-4,5-diphosphate (PtdIns(4,5)P_2) from Cayman Chemical. All SPR measurements were performed at 24 °C in 20 mM Tris–HCl, pH 7.4, containing 0.16 M NaCl using a lipid-coated L1 chip in the BIACORE X system as described previously.[28] The control channel (flow cell 1; FC1) was coated by POPC/POPS (80:20 mol %) unilamellar vesicles, and the active channel (flow cell 2; FC2) by POPC/POPS/PtdIns(3,4,5)P_3 (77:20:3 mol %). The inhibitory effects on PtdIns(3,4,5)P_3 binding of each compound was determined by comparison of response resonance unit (RU) between Btk PH domain with and without small chemicals. For the lipid binding inhibition screening of small chemicals, 200 nM of Btk PH domain alone and mixed with 100 μM of each chemical were tested by SPR. The chemical showing the highest decrease of PtdIns(3,4,5)P_3 binding were selected for further experiments. The selected lipid binding inhibition candidates were further investigated by SPR with various concentrations, 10 to 500 μM to determine inhibitory activity. The decrease response unit (RU) value of each injection with various concentrations was calculated to the inhibition efficiency (percentage,%).

All docking experiments were performed on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 (Tripos).[29] The docking radius was set to 6.5 Å, and the residues for docking were selected. The 3D structure of Btk PH domain determined in 1999 by crystallography was deposited in Protein Data Bank as 1b55.pdb.[30] It was deposited as a homodimer with a ligand, inositol tetraphosphates (IP4), so the 169 residue of chain A was used in this study. The ligand, IP4, in Btk PH domain was removed by Sybyl program to generate apo-protein, and then it was processed by energy minimization. The apo-protein was compared with the original structure of Btk PH domain to evaluate any differences. Since the Sybyl program provides flexible docking procedure, the binding pocket was defined first. The docking radius was set to 6.5 Å, and the residues for docking were selected; Lys12, Ser14, Gln15, Lys17 Lys18, Ser21, Asn24, Arg28 and Tyr39, Gln16, Pro22, and Lys53. The docking process was iterated 30 times, resulting 30 poses. The complex exhibiting the best docking score and docking pose was selected and analyzed in order to elucidate the

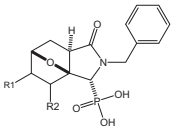
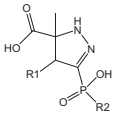
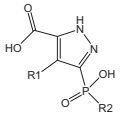
Chemical No.	Structure	R1	R2	Relative inhibitory activity (%)*
1		H	H	38
2		OH	OH	13
3		O-PO_3H_2	OH	11
4		H	H	71
5		H	OCH_3	52
6		H	OC_2H_5	41
7		COOH	OH	64
8		H	OH	95
9		H	OCH_3	48
10		H	OC_2H_5	37
11		COOH	OC_2H_5	34

Figure 1. Structures of small chemicals used in this study, chemical 1–11, and relative lipid binding inhibitory efficiency with 100 μM of each chemical. *Relative inhibitory activity of each chemical was determined by in vitro SPR measurements. It was calculated from decrease of response resonance unit by addition of 100 μM of each chemical.