Biochemical and Biophysical Research Communications 377 (2008) 23–28

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Crystal structure of the Bruton's tyrosine kinase PH domain with phosphatidylinositol

Kazutaka Murayama [a,b], Miyuki Kato-Murayama [b], Chiemi Mishima [b], Ryogo Akasaka [b], Mikako Shirouzu [b], Yasuhisa Fukui [c,1], Shigeyuki Yokoyama [b,d,*]

[a] Graduate School of Biomedical Engineering, Tohoku University, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
[b] Systems and Structural Biology Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan
[c] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[d] Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

ARTICLE INFO

Article history:
Received 3 September 2008
Available online 20 September 2008

Keywords:
Complex structure
Pleckstrin homology domain
Phosphatidylinositol
Membrane targeting
Dimerization

ABSTRACT

Bruton's tyrosine kinase (Btk) of the Tec family possesses a Pleckstrin homology (PH) domain, which is responsible for plasma membrane targeting. In this study, the crystal structure of the Btk PH domain in complex with dibutylyl-phosphatidylinositol-3,4,5-triphosphate was determined. The structure revealed that the Btk PH domain forms a homodimer and that each molecule binds phosphatidylinositol in the binding pocket. The side chain of Lys18 within a Btk-specific insertion in the β1–β2 loop is able to form a hydrogen bond with the diacylglycerol moiety of phosphatidylinositol. The other Btk-specific insertion in the β5–β6 loop constitutes the dimerization interface. Thus, the modes of phosphatidylinositol recognition and Btk PH domain dimerization are distinct from those of other PH domains.

Bruton's tyrosine kinase (Btk) gene mutations cause the immunodeficiency disease X-linked agammaglobulinemia in humans and X-linked immunodeficiency (Xid) in mice [1,2]. The disease is the result of a B-lymphocyte developmental defect, in which Btk-dependent signal transduction pathways are inactivated, and B cells remain at the pre-B cell stage. Btk belongs to the Tec family of non-receptor protein tyrosine kinases, whose members, Itk, Rlk, Tec, Btk, and Bmx, share a common domain construction [3]. In its N-terminal region, Btk possesses a Pleckstrin homology (PH) domain characteristically followed by the Tec homology (TH) domain. The TH domain contains a Zn^{2+} binding region, known as the Btk motif, and a proline-rich region. The C-terminal region contains a Src homology (SH) 2 domain, an SH3 domain, and a tyrosine kinase domain (Fig. 1A).

Lyn activates phosphatidylinositol 3-kinase following B cell receptor stimulation, and then phosphatidylinositol-3,4,5-triphosphate (PtdIns(3,4,5)P_3) is produced. Subsequently, Btk is recruited to the plasma membrane, where it interacts with PtdIns(3,4,5)P_3 [3]. The PH domain is responsible for binding with phosphoinositides and is important for the regulation of membrane recruitment [4,5]. The Btk PH domain recognizes PtdIns(3,4,5)P_3 more specifically than other phosphatidylinositols [6]. The crystal structures of the complexes between the PH domains and various ligands have revealed their specific binding in canonical [7–14] and non-canonical [15,16] manners. The crystal structure of the complex of the Btk PH domain with D-*myo*-inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P_4) revealed that the Btk PH domain recognizes Ins(1,3,4,5)P_4 in the canonical manner [7]. Most of such ligands are water soluble inositol phosphates, and discussions of phosphatidylinositol recognition have focused on the phosphate groups of the inositol ring. In addition, the affinity of the Btk PH domain for PtdIns(3,4,5)P_3 varies with different lipid lengths [6]. This implies that the Btk PH domain recognizes not only inositol phosphates but also the diacylglycerol moiety and/or the lipid. All of the crystal structures of the Btk PH domain have been determined as dimers, although it may be a monomer in solution [17]. Therefore, it is possible that the Btk PH domain dimerizes upon membrane binding and that its phosphatidylinositol recognition is important.

In the present study, we determined the crystal structure of a Btk fragment consisting of the PH domain and the Btk motif in complex

Abbreviations: SH, Src homology; PH, Pleckstrin homology; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-triphosphate; Ins(1,3,4,5)P_4, D-*myo*-inositol 1,3,4,5-tetrakisphosphate

* Corresponding author. Address: Systems and Structural Biology Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan. Fax: +81 45 503 9195.
E-mail address: yokoyama@biochem.s.u-tokyo.ac.jp (S. Yokoyama).
[1] Present address: Institute of Medical Chemistry, Hoshi University, 2-4-4-1 Ebara, Shinagawa, Tokyo 142-8501, Japan.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.09.055

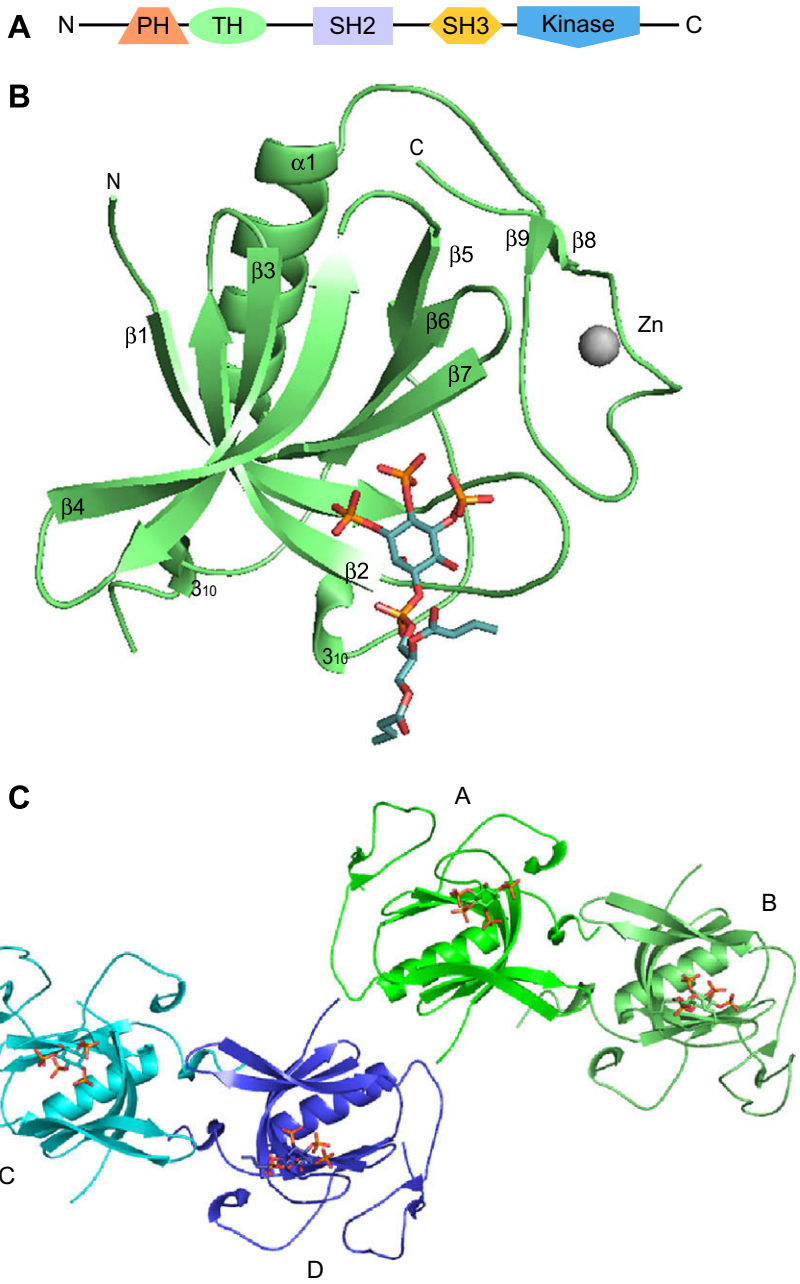

Fig. 1. (A) Schematic presentation of the domain architecture of Btk. (B) Ribbon representation of the crystal structure of the Btk PH domain (monomer structure, molecule B). The phosphatidylinositol molecule and the Zinc ion are drawn in a stick model and gray sphere, respectively. (C) Crystal structures of the Btk PH domains in the asymmetric unit.

with dibutylyl-phosphatidylinositol-3,4,5-triphosphate (C4PtdIns(3,4,5)P_3), to clarify the PtdIns(3,4,5)P_3 recognition and dimerization mechanisms. The electron density of C4PtdIns(3,4,5)P_3 was observed in the ligand binding sites of the two PH domain molecules, suggesting that the diacylglycerol moieties are involved in phosphatidylinositol recognition by the Btk PH domain dimer.

Materials and methods

Expression and purification. The Btk PH domain was expressed in *Spodoptera frugiperda* Sf9 insect cells using the baculovirus expression system. The cells were collected by centrifugation. After sonication, the insoluble components were removed by centrifugation (16,000*g*) for 60 min. The supernatant was loaded onto a HiTrap SP (GE Healthcare) column, equilibrated with 20 mM Tris–HCl buffer (pH 7.0) containing 100 mM NaCl and 1 mM DTT. The Btk PH domain was eluted by a linear gradient of 0–1 M NaCl. Btk PH domain-containing fractions were pooled and then loaded onto a Mono S (GE Healthcare) column, equilibrated with 20 mM Tris–HCl buffer (pH 7.0) containing 100 mM NaCl and 1 mM DTT, which was eluted by a linear gradient of 0–1 M NaCl. Fractions containing the protein were pooled and subjected to gel-filtration on a HiLoad Superdex75 (GE Healthcare) column equilibrated with 20 mM Tris–HCl buffer (pH 7.0), containing 100 mM NaCl and 1 mM DTT. Finally, the protein was concentrated to ~12.6 mg/ml with an Amicon ultra filter (Millipore).

Crystallization and data collection. The purified Btk PH domain and C4PtdIns(3,4,5)P_3 were co-crystallized by the hanging-drop vapor-diffusion method. The protein solution, at a final protein concentration of 6.3 mg/ml, was mixed with 0.5 mg/ml