Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 364 (2007) 111–117
BBRC
www.elsevier.com/locate/ybbrc

Interdomain A is crucial for ITAM-dependent and -independent regulation of Syk

Takahiro Adachi [a,b,c,*], Jürgen Wienands [d], Takeshi Tsubata [a,b,c], Tomohiro Kurosaki [e]

[a] *Laboratory of Immunology, Biomedical Science, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan*
[b] *Department of Immunology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan*
[c] *CREST, JST 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan*
[d] *Department of Cellular and Molecular Immunology, Medical Faculty of the Georg-August-University of Göttingen, Göttingen 37073, Germany*
[e] *Laboratory for Lymphocyte Differentiation, RIKEN Research Center for Allergy and Immunology, Yokohama, Kanagawa 230-0045, Japan*

Received 21 September 2007
Available online 2 October 2007

Abstract

Non-receptor type protein tyrosine kinase (PTK) Syk is essential for the signaling via the B cell antigen receptor (BCR). Upon BCR crosslinking, Syk is recruited via its tandem SH2 domains to tyrosine-phosphorylated Ig-α/Ig-β constituting components of BCR, and is then activated. The interdomain A lying between the two SH2 domains is highly conserved among different species of Syk and between Syk and ZAP-70. The mutant Syk carrying a deletion in the interdomain A (Δ140–159) became phosphorylated regardless of BCR ligation and did not induce Ca^{2+} mobilization upon crosslinking of BCR. Furthermore, in vitro binding assay revealed that deletion of a part of the interdomain A abolished its binding activity to phosphorylated Ig-α/Ig-β. These results indicate that the interdomain A of Syk is required for activation of Syk by binding to the phosphorylated Ig-α/Ig-β upon BCR ligation and inhibition of spontaneous activation at the resting state.
© 2007 Elsevier Inc. All rights reserved.

Keywords: Tyrosine kinase; BCR; Syk; Signaling; B lymphocyte

Syk is a non-receptor type protein tyrosine kinase (PTK) which is present in most hematopoietic cells including B cells, mast cells, macrophages, and T cells [1]. The overall structure of Syk is highly conserved with that of ZAP-70, another member of the Syk PTK family expressed specifically in T and NK cells [1]. The Syk family PTKs contain following domains from the N- to the C-terminus (Fig. 1): (i) the N-terminal SH2 domain; (ii) the interdomain A; (iii) the C-terminal SH2 domain; (iv) the interdomain B; (v) the catalytic domain; and (vi) a short C-terminal extension. Syk is involved in signal transduction through BCR and FcεRI [1]. The ligation of the B cell antigen receptor (BCR) or the high affinity receptor for IgE (FcεRI) initiates rapid phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) [2] in Ig-α and Ig-β in B cells, or FcεRIβ and FcεRIγ in mast cells, respectively, by Src-family PTKs such as Lyn. The phosphorylated ITAM binds to the tandem SH2 domains of Syk resulting in recruitment of Syk to the ligated BCR or FcεRI. Syk is then phosphorylated and activated in the receptor complexes [3]. The activated Syk in turn phosphorylates itself including tyrosine residues in the interdomain B, leading to recruitment and activation of downstream effector molecules such as Vav and PLC-γ1 [4,5]. DT40 chicken B cells in which the syk gene is disrupted by gene targeting fail to generate Ca^{2+} mobilization upon BCR crosslinking [6], indicating that Syk is essential

Abbreviations: FcεRI, high affinity IgE receptor; ITAM, immunoreceptor tyrosine-based activation motif; PLC, phospholipase C; PTK, protein tyrosine kinase; Ab, antibody; mAb, monoclonal antibody; BCR, B cell antigen receptor.

* Corresponding author. Address: Department of Immunology, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan. Fax: +81 3 5803 5818.
E-mail address: tadachi.imm@mri.tmd.ac.jp (T. Adachi).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.09.100

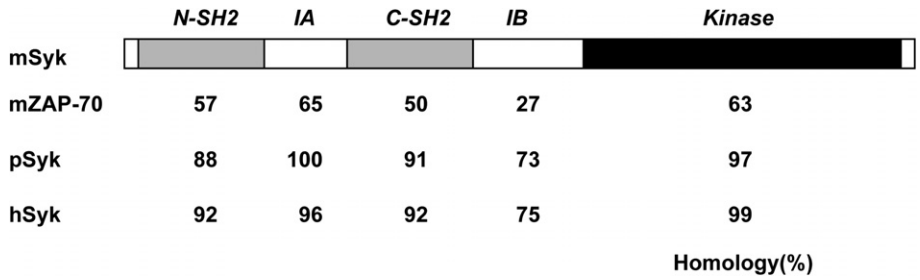

Fig. 1. Schematic representation of domain structure of the mouse Syk and homologies of the amino acid sequences of various domains of mouse Syk (mSyk) to those of porcine and human Syk (pSyk and hSyk, respectively) and mouse ZAP-70 (mZAP-70). Homologies (percentage) are indicated. Abbreviations: N, N-terminal; IA, interdomain A; C, C-terminal; IB, interdomain B.

in BCR signaling. Taken together, Syk is crucial for signaling through BCR and FcεRI.

Each domain of Syk appears to carry distinct functions. Indeed, the tandem SH2 domains bind the doubly phosphorylated ITAM and are essential for the recruitment of Syk to BCR or FcεRI [1]. The interdomain B contains autophosphorylation sites and plays a role in recruiting downstream signaling molecules [5,7–9]. Moreover, an isoform of Syk (SykB) lacking 23 amino acids in the interdomain B fails to bind to the ITAM. Keshvara et al. have demonstrated that replacement of the tyrosine residue in the N-terminal part of the interdomain A reduces the kinase activity of Syk [10], indicating that the interdomain A plays a role in the signaling function of Syk. Furthermore, crystal structure of Syk and ZAP-70 reveals that the interdomain A forms a helical coiled-coil structure and is inferred to provide appropriate conformation of the tandem SH2 for the binding to the diphosphorylated ITAM [11,12]. However, roles of the interdomain A in the signaling function of Syk are not fully understood. Here, we demonstrate that the interdomain A of Syk is highly conserved among different species, and C-terminal part of the interdomain A but not tyrosine residue is critical for Ca^{2+} mobilization by BCR signaling and the regulation of kinase activity at the resting state. Thus, C-terminal part of the interdomain A may play an essential role in the signaling function of Syk.

Materials and methods

Cells, culture, and transfection. J558Lμm3, DT40, and WEHI-231 cells were cultured as described previously [6,13,14]. To generate an expression plasmid for Syk126F, pApuro Syk126F, site-directed mutagenesis was carried out as described previously [15] using an oligonucleotide (5′-TC CGGGAATTCGTGAAACAG-3′) and the porcine Syk cDNA. The mutagenesis was confirmed by DNA sequencing. For construction of pApuro SykΔ , two XhoI sites were introduced into the Syk cDNA by site-directed mutagenesis using two oligonucleotides (Xho-2; 5′-TCAAGCC CTCGAGCAGGCAA-3′ and Xho-3; 5′-CCACAGCCCTCGAGAA AATGCCC-3′) followed by deletion of the XhoI fragment. pApuro Syk 126F or pApuro SykΔ was transfected into Syk-deficient DT40 cells as described previously [6]. pMXsIN SykΔ or pMXsIN Syk was constructed by introducing SykΔ or wild-type Syk cDNA into a retrovirus vector pMXs IN and transfected into J558Lμm3 and WEHI-231 cells [16].

In vitro binding assay. pGEX-SYK [N+C] carrying a fusion gene of GST and tandem SH2 of the human Syk including the interdomain A was described previously [17]. pGEX-SYK M1 was generated by mutating pGEX-SYK [N+C] using two oligonucleotides (SM-1; 5′-AGCCT CAGCTCGAGAAGCTG-3′ and SM-2; 5′-ACCACAGCCCTCGA GAAAATGCCT-3′) followed by deletion of the XhoI fragment. pGEX-SYK M2 was also generated by site-directed mutagenesis using an oligonucleotide (SM-3; 5′-CACAGCCCATATGCCTTGGT-3′) and pGEX- SYK [N+C]. pGEX-SYK [N+C] and its mutants were introduced into *Escherichia coli* and GST fusion proteins were prepared as described previously [18]. Five micrograms of GST-fusion proteins was incubated with cell lysates of pervanadate-activated WEHI-231 containing various phosphorylated proteins [19] and glutathione Sepharose 4B beads (Amersham Biosciences, Uppsala, Sweden) for 30 min at 4 °C. Alternatively, anti-Ig-β mAb HM79 [20] together with protein G beads (Amersham Biosciences) were incubated with cell lysates of pervanadate-activated WEHI-231. The beads were washed by PBS three times and added in SDS–PAGE sample buffer. Then proteins bound to the beads were subjected to Western blot analysis.

Western blot analysis. Cells were collected and lysed in SDS–PAGE sample buffer and proteins were separated by SDS–PAGE under reducing conditions. Membranes were incubated with rabbit anti-GST-Syk Ab [20], rabbit anti-SLP-65 Ab [21], mouse anti-phospotyrosine mAb 4G10 (Upstate Biotechnology, Lake Placid, NY), or anti-β-tubulin mAb (Seikagaku Kogyo, Tokyo, Japan), followed by incubation with peroxidase-conjugated sheep anti-mouse Ig Ab (Amersham Biosciences), or goat anti-rabbit Ig Abs (Cell Signaling Technology, Beverly, MA). Proteins were then visualized by Chemi-Lumi One (Nacalai Tesque, Kyoto, Japan).

Ca^{2+} mobilization analysis. Ca^{2+} mobilization analysis was performed as described previously [6].

Results

The interdomain A of Syk is conserved among different species and with that of ZAP70

Comparison of each domain of Syk among different species (mouse, human, and porcine) revealed striking conservation of the interdomain A (97–100%), even higher degree than either the N-terminal or C-terminal SH2 domain (88–92%) (Fig. 1) [22–24]. Furthermore, the interdomain A is conserved more strikingly (65%) between mouse Syk and ZAP-70 [25] than SH2 domains (50–57%). The C-terminal half of the interdomain A is almost identical between Syk and ZAP-70 (data not shown). In addition, the interdomain A of Syk consists of 60 amino acid residues in mouse, human, and pig, whereas there are slight differences in the length of SH2, the interdomain B and catalytic domains among those species. These findings suggest that the interdomain A plays an important role in the function of Syk.

Syk carrying a deletion in the interdomain A fails to regulate kinase activity at the resting state

To assess the role of the interdomain A in the signaling function of Syk, either the mutant Syk lacking a part of the interdomain A (SykΔ) or wild-type Syk (Fig. 2A) is expressed in mouse myeloma J558Lμm3 cells which carry IgM-BCR specific for (4-ydorxy-3-nitrophenyl)acetyl (NP). In these cells, SykΔ became highly phosphorylated without BCR ligation while wild-type Syk did not get phosphorylated (Fig. 2B). Furthermore, mouse lymphoma line WEHI-231 cells expressing SykΔ also exhibit phosphorylation of SykΔ at the resting state. These results suggest that the interdomain A may contribute to the intramolecular regulation of kinase activity at the resting state.

Syk carrying a deletion in the interdomain A fails to transduce BCR signaling

We examined signaling function of Syk mutants in J558Lμm3 cells upon BCR stimulation. Although SykΔ became phosphorylated without BCR stimulation, SykΔ did not augment phosphorylation of cellular substrates upon BCR ligation by antigen NP-BSA in J558Lμm3 cells (Fig. 2C). In contrast, wild-type Syk enhanced phosphorylation of cellular substrates upon BCR ligation.

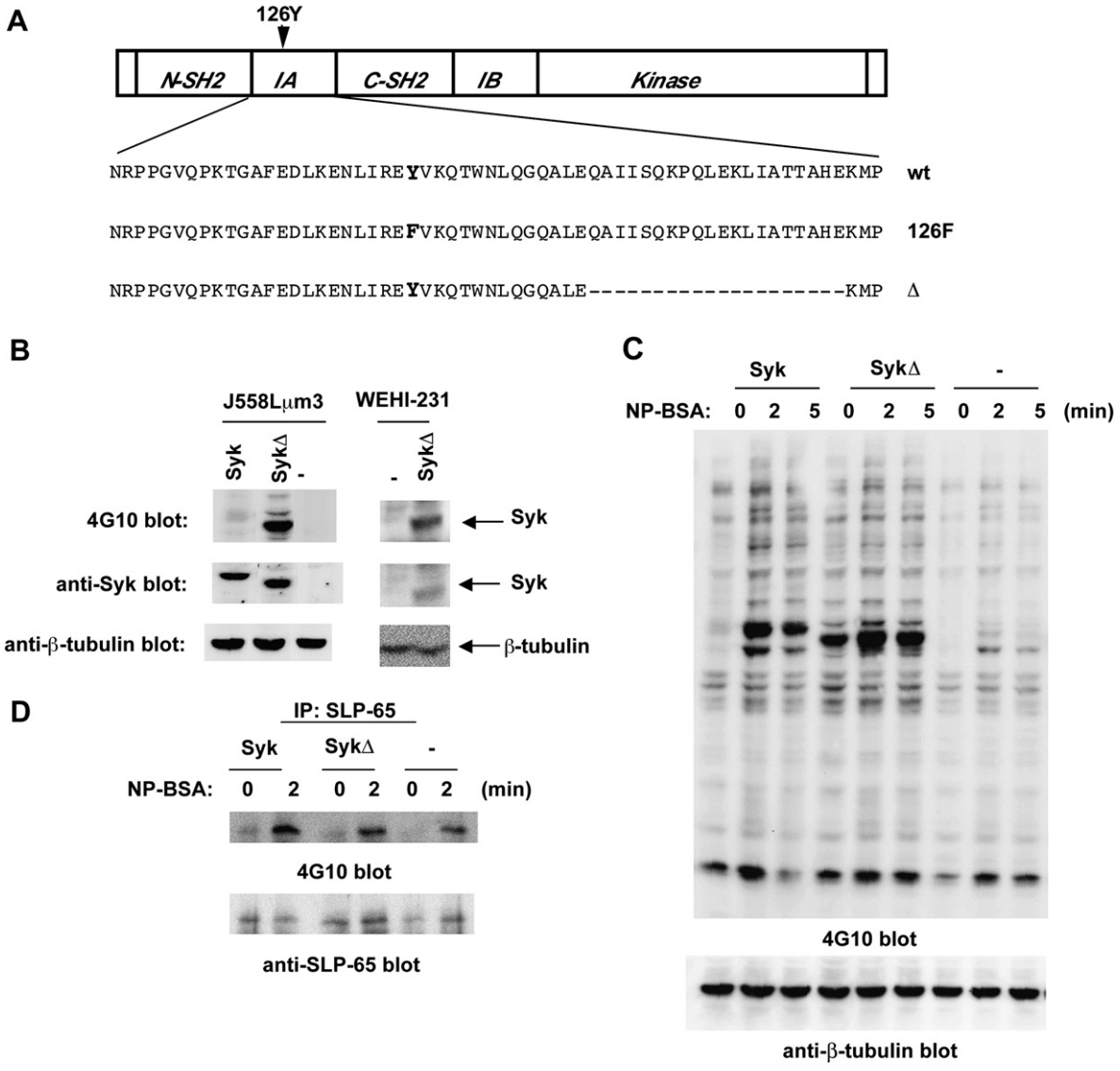

Fig. 2. Syk lacking a part of the interdomain A fails to regulate its kinase activity. (A) Schematic representation of porcine Syk mutants. The autophosphorylation tyrosine residue in the interdomain A is indicated by a bold-face letter. (B) Western blot analysis of tyrosine phosphorylation of porcine Syk and its mutants in J558Lμm3 and WEHI-231 cells. Whole cell lysates were probed with anti-phosphotyrosine mAb 4G10 and anti-Syk Ab. To verify equal loading, the membrane was reprobed with anti-β-tubulin mAb. (C and D) Phosphorylation of cellular substrates upon BCR stimulation of J558Lμm3 cells expressing Syk or its mutants. Cells were stimulated with 0.2 μg/ml NP-BSA for the indicated time periods at 37 °C. Whole cell lysates (C) and immunoprecipitates with anti-SLP-65 Ab (D) were probed with anti-phosphotyrosine mAb 4G10. To verify equal loading, the membranes were reprobed with anti-β-tubulin mAb (C) and with anti-SLP-65 Ab (D).

Furthermore, to assess the activation of Syk upon BCR stimulation, we examined phosphorylation of an adaptor protein SLP-65 (also known as BLNK and BASH) which is a dominant substrate of Syk [21,26,27]. In consistent with phosphorylation of total cell lysates, wild-type Syk but not SykΔ enhanced phosphorylation of SLP-65 upon BCR ligation (Fig. 2D), indicating that SykΔ fails to phosphorylate SLP-65 upon BCR ligation. Thus, a deletion of a part of the interdomain A causes not only loss of self-regulation of kinase activity at the resting state but also inability to mediate BCR signaling.

To evaluate the role of the interdomain A in the signaling function of Syk more precisely, we reconstituted Syk and its mutants in Syk-deficient chicken DT40 B cells and examined Ca^{2+} mobilization upon BCR stimulation. In addition to SykΔ, we constructed an expression plasmid for a mutated porcine Syk in which Y126 was replaced by phenylalanine (Syk126F), as Y130 of mouse Syk corresponding to Y126 of porcine Syk is one of the autophosphorylation sites [28] (Fig. 2A) because phosphorylation at this tyrosine has been shown to augment its kinase activity [10]. Expression plasmids for wild-type porcine Syk, Syk126F, and SykΔ were introduced into Syk-deficient DT40 cells. Western blot analysis revealed that most transfectants expressed similar amounts of Syk proteins (Fig. 3A). When we crosslinked BCR by treatment with anti-Ig Ab, DT40 transfectants expressing wild-type porcine Syk instead of chicken Syk showed efficient Ca^{2+} mobilization (Fig. 3B), whereas Ca^{2+} response was not detected in Syk-deficient DT40 cells (data not shown) as described previously [6]. DT40 expressing Syk126F showed Ca^{2+} mobilization almost equivalent to that of DT40 expressing wild-type Syk, indicating that Y126 is not crucial for Ca^{2+} signaling. In contrast, Ca^{2+} mobilization was undetectable in DT40 expressing SykΔ. This result indicates that the C-terminal part of the interdomain A is critical for the signaling function of Syk.

Syk carrying a deletion in the interdomain A fails to associate with phosphorylated Ig-α/Ig-β

Interaction with the phosphorylated Ig-α/Ig-β is essential for activation of Syk upon BCR crosslinking. To test whether the mutant Syk lacking a part of the interdomain A carries defects in interaction with Ig-α/Ig-β, we prepared GST fusion proteins containing the tandem SH2 domains including the interdomain A with or without deletions in the interdomain A (Fig. 4A). When we incubated GST fusion proteins with pervanadate-treated WEHI-231 cell lysates in which tyrosine-phosphorylation of cellular proteins was induced, GST fusion protein containing the wild-type interdomain A precipitated phosphotyrosine-containing proteins of 33–36 kDa and 37–39 kDa which appear Ig-α and Ig-β, respectively, and 150 kDa which is likely to be CD22 (Fig. 4B) in agreement with previous reports [17,29]. Although similar amounts of GST-fusion

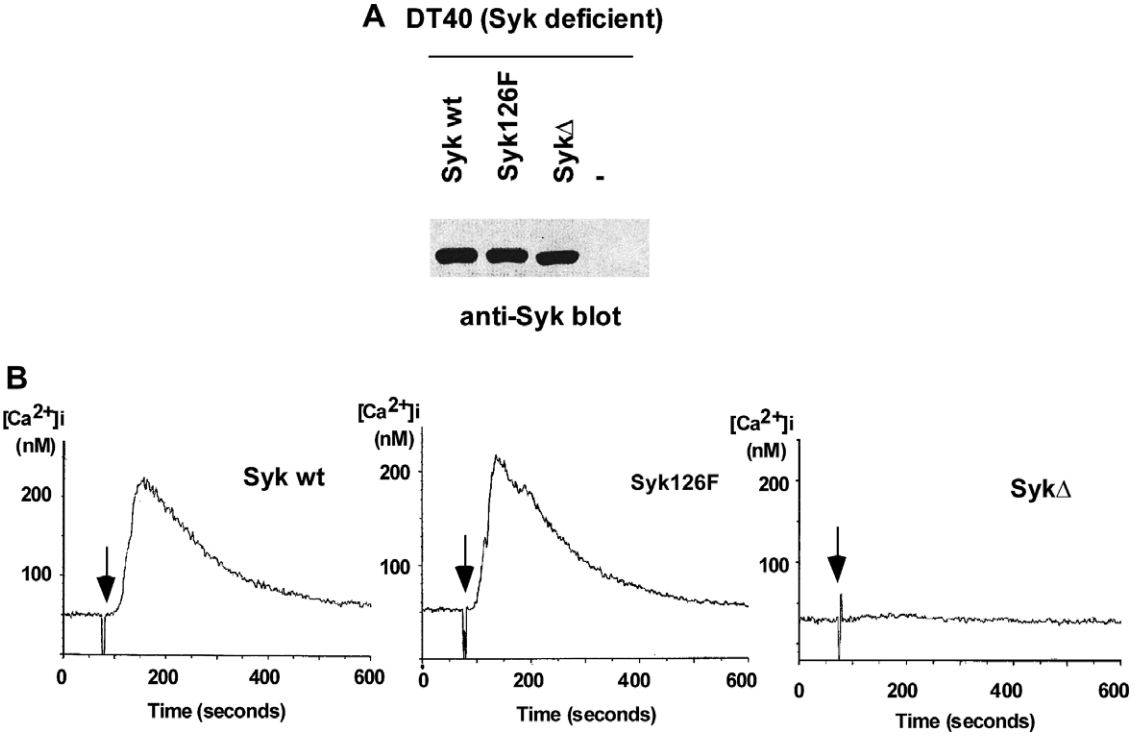

Fig. 3. Syk lacking a part of the interdomain A fails to induce Ca^{2+} mobilization upon BCR ligation. (A) Expression of porcine Syk or its mutants in DT40 cells. Whole cell lysates were probed with anti-Syk Ab. (B) Ca^{2+} mobilization upon BCR stimulation of DT40 cells expressing Syk or its mutants. Syk-deficient DT40 cells were transfected with wild-type Syk, Syk126F, or SykΔ. Transfectants were loaded with fura-2/AM, and stimulated with anti-chicken IgM (M4, 1 μg/ml), followed by analysis of $[Ca^{2+}]_i$ using fluorimetry.

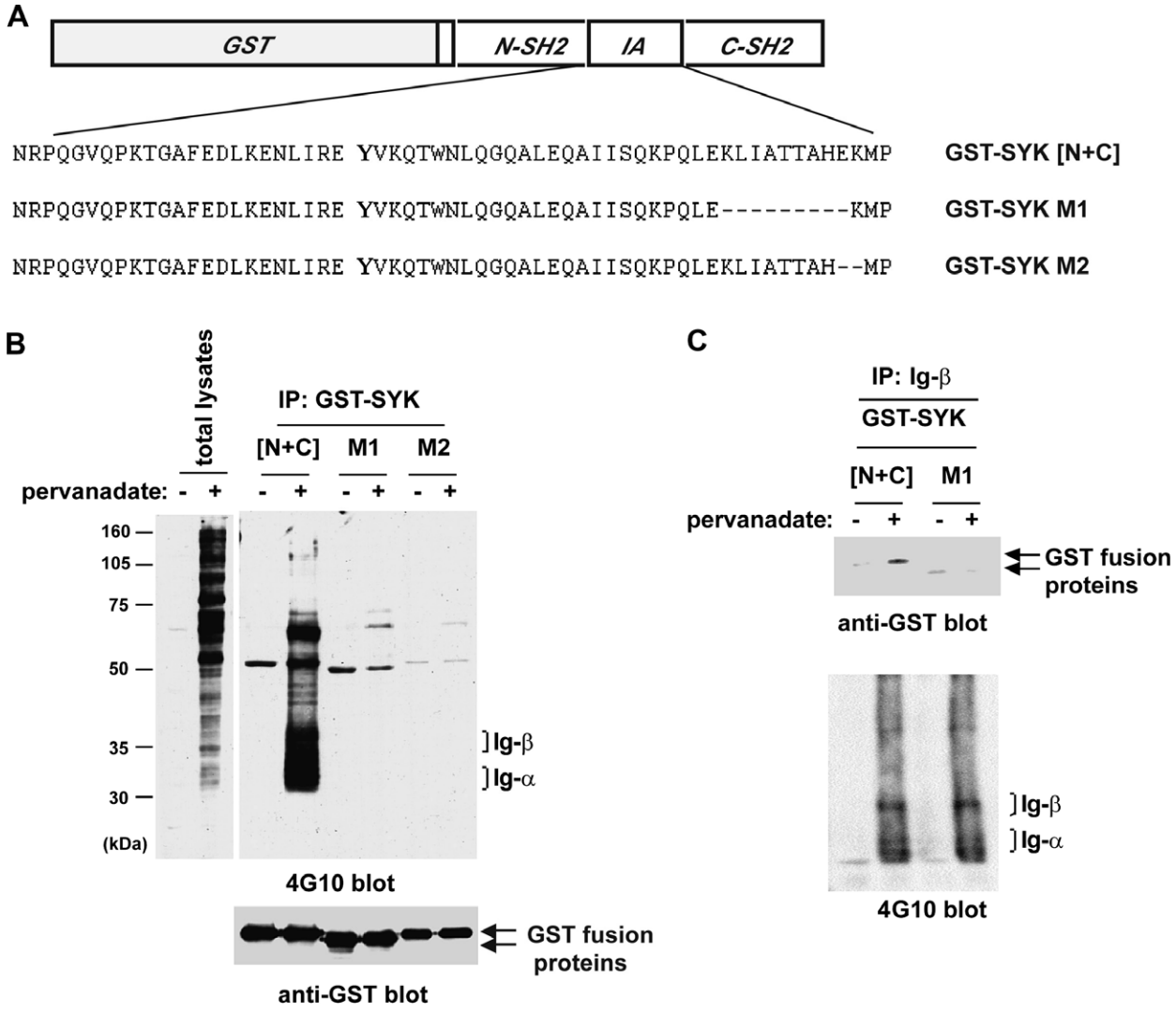

Fig. 4. Syk lacking a part of the interdomain A fails to associate with phosphorylated Ig-α/Ig-β. (A) Schematic representation of GST-SYK [N+C] and its mutants. (B and C) Binding of GST-SYK [N+C] and its mutants to phosphotyrosine-containing proteins. WEHI-231 cells (2×10^6) were either unstimulated or stimulated with 25 μM pervanadate for 5 min at 37 °C. Cells were lysed in 1% Triton X-100 lysis buffer, and cleared cell lysates were incubated with 5 μg of GST-SYK [N+C], GST-SYK M1 or GST-SYK M2 followed by incubation with glutathione beads (B). Alternatively, cleared cell lysates were incubated with anti-Ig-β mAb together with protein G beads (C). Proteins bound to the beads were analyzed by Western blotting with anti-phosphotyrosine mAb 4G10 or anti-GST Ab. As a control, total cell lysates were analyzed in parallel. The positions for GST-SYK fusion proteins, Ig-α, and Ig-β are indicated.

proteins were precipitated, extremely small amounts of phosphotyrosine-containing proteins were coprecipitated with the GST fusion proteins GST-SYK M1 and M2 lacking amino acid residues corresponding to 151–159 and 159–160 of porcine Syk, respectively. Furthermore, when the Ig-α/Ig-β heterodimer was precipitated by anti-Ig-β mAb, GST-SYK (wild-type), but not M1 was coprecipitated with the phosphorylated Ig-α/Ig-β heterodimer (Fig. 4C). These results indicate that the interdomain A is critical for the recruitment of Syk to the BCR via Ig-α/Ig-β.

Discussion

We have assessed the role of the interdomain A in the signaling function of Syk by using mutant Syk in which Y126 is replaced by phenylalanine and that carries a deletion in the C terminal part of the interdomain A. Syk126F is capable of replacing wild-type Syk in Ca^{2+} signaling via BCR. Y126 is thus dispensable for Ca^{2+} mobilization mediated by Syk, although the replacement of Y130 of mouse Syk corresponding to Y126 of porcine Syk reduces the kinase activity and retards release of Syk from BCR complex [10]. Remaining kinase activity of Syk126F may be sufficient for inducing Ca^{2+} mobilization upon BCR ligation. In contrast, we demonstrate that Syk lacking a part of the interdomain A fails to regulate its kinase activity at the resting state and loses ability to mediate BCR signaling, indicating that the interdomain A is essential for the signaling function of Syk. Moreover, Syk lacking a part of the interdomain A fails to associate with phosphorylated

Ig-α/Ig-β. Recruitment of Syk to the phosphorylated ITAM of Ig-α/Ig-β is essential for the activation of Syk [1]. The interdomain A is thus essential for the signaling function of Syk for the recruitment of Syk to Ig-α/Ig-β upon BCR signaling and the regulation of kinase activity at the resting state.

Upon signaling via BCR, TCR or FcεRI, Syk or ZAP70 is recruited to the ITAM in the components of the receptors and then activated. This interaction requires association of both of the SH2 domains of Syk/ZAP70 with the doubly phosphorylated tyrosines in the ITAM [3,30]. Our data indicate that the interdomain A is involved in association of Syk with the ITAM. The interdomain A may contribute to the structural conformation which facilitates or stabilizes the interaction between the SH2 domains and the phosphorylated ITAMs. This is supported by the findings on the crystal structure of ZAP-70 and Syk in complex with a doubly phosphorylated peptide derived from the ITAM sequence of the TCR ζ-chain and TCR ε-chain, respectively. Indeed, the analysis on the crystal structure revealed that the interdomain A forms a coiled-coil loop by antiparallel α-helices and provides an interface between the two SH2 domains [11,12]. This result suggests that the interdomain A not only functions as a hinge but also provides an important structural information in which it keeps two SH2 domains with an appropriate distance and angle for binding to the precisely spaced phosphotyrosines in the ITAM. Probably, deletion of the C-terminal part of the interdomain A alters the coiled-coil structure, resulting that both of the tandem SH2 domains no longer bind to the each of the two phosphotyrosines in the ITAM simultaneously.

In the resting cells, kinase activity of Syk is inhibited by N-terminal part of Syk including both SH2 [31]. Tandem SH2 including the interdomain A plays a role not only in ITAM binding but also in inhibition of kinase activity probably due to intramolecular interaction between the catalytic domain and tandem SH2. Recently, the C-terminal SH2 has been shown to be sufficient for the inhibition of spontaneous activation of Syk by using a Syk mutant lacking the N-terminal SH2 and the interdomain A [32]. However, we showed here that a Syk mutant carrying a part of deletion in the interdomain A also lose ability to regulate kinase activity in the resting cells. Probably, the interdomain A may contribute to whole conformational structure of Syk and allow the C-terminal SH2 domain to interact with the catalytic domain in the resting cells, resulting in inhibition of its kinase activity. Analysis of crystal structure of the complete Syk protein without ITAM peptides may be needed to clarify the regulation of intramolecular interaction.

In summary, we found striking conservation of the Syk interdomain A among various species and between Syk and ZAP-70. Our data clearly demonstrate the essential role of the interdomain A in the signaling function of Syk both during BCR stimulation in the ITAM-dependent manner and in the resting state in the ITAM-independent manner.

Acknowledgments

We are grateful to Dr. T. Kitamura (The University of Tokyo) for reagents, and Ms. M. Kurosaki (RIKEN) and Mr. K. Oshima (Nagoya University) for technical assistance.

References

[1] M. Turner, E. Schweighoffer, F. Colucci, J.P. Di Santo, V.L. Tybulewicz, Tyrosine kinase SYK: essential functions for immunoreceptor signalling, Immunol. Today. 21 (2000) 148–154.
[2] M. Reth, Antigen receptor tail clue, Nature 338 (1989) 383–384.
[3] T. Kimura, H. Sakamoto, E. Appella, R.P. Siraganian, Conformational changes induced in the protein tyrosine kinase p72syk by tyrosine phosphorylation or by binding of phosphorylated immunoreceptor tyrosine-based activation motif peptides, Mol. Cell. Biol. 16 (1996) 1471–1478.
[4] M. Deckert, D.S. Tartare, C. Couture, T. Mustelin, A. Altman, Functional and physical interactions of Syk family kinases with the Vav proto-oncogene product, Immunity 5 (1996) 591–604.
[5] C.L. Law, K.A. Chandran, S.P. Sidorenko, E.A. Clark, Phospholipase C-gamma1 interacts with conserved phosphotyrosyl residues in the linker region of Syk and is a substrate for Syk, Mol. Cell. Biol. 16 (1996) 1305–1315.
[6] M. Takata, H. Sabe, A. Hata, T. Inazu, Y. Homma, T. Nukada, H. Yamamura, T. Kurosaki, Tyrosine kinases Lyn and Syk regulate B cell receptor-coupled Ca^{2+} mobilization through distinct pathways, EMBO J. 13 (1994) 1341–1349.
[7] J.J. Hong, T.M. Yankee, M.L. Harrison, R.L. Geahlen, Regulation of signaling in B cells through the phosphorylation of Syk on linker region tyrosines. A mechanism for negative signaling by the Lyn tyrosine kinase, J. Biol. Chem. 277 (2002) 31703–31714.
[8] J. Zhang, E. Berenstein, R.P. Siraganian, Phosphorylation of Tyr342 in the linker region of Syk is critical for FcεRI signaling in mast cells, Mol. Cell. Biol. 22 (2002) 8144–8154.
[9] M. Simon, L. Vanes, R.L. Geahlen, V.L. Tybulewicz, Distinct roles for the linker region tyrosines of Syk in FcεRI signaling in primary mast cells, J. Biol. Chem. 280 (2005) 4510–4517.
[10] L.M. Keshvara, C. Isaacson, M.L. Harrison, R.L. Geahlen, Syk activation and dissociation from the B-cell antigen receptor is mediated by phosphorylation of tyrosine 130, J. Biol. Chem. 272 (1997) 10377–10381.
[11] M.H. Hatada, X. Lu, E.R. Laird, J. Green, J.P. Morgenstern, M. Lou, C.S. Marr, T.B. Phillips, M.K. Ram, K. Theriault, a.l. et, Molecular basis for interaction of the protein tyrosine kinase ZAP-70 with the T-cell receptor, Nature 377 (1995) 32–38.
[12] K. Futterer, J. Wong, R.A. Grucza, A.C. Chan, G. Waksman, Structural basis for Syk tyrosine kinase ubiquity in signal transduction pathways revealed by the crystal structure of its regulatory SH2 domains bound to a dually phosphorylated ITAM peptide, J. Mol. Biol. 281 (1998) 523–537.
[13] T. Adachi, J. Wienands, C. Wakabayashi, H. Yakura, M. Reth, T. Tsubata, SHP-1 requires inhibitory co-receptors to down-modulate B cell antigen receptor-mediated phosphorylation of cellular substrates, J. Biol. Chem. 276 (2001) 26648–26655.
[14] T. Tsubata, J. Wu, T. Honjo, B-cell apoptosis induced by antigen receptor crosslinking is blocked by a T-cell signal through CD40, Nature 364 (1993) 645–648.
[15] T.A. Kunkel, J.D. Roberts, R.A. Zakour, Rapid and efficient site-specific mutagenesis without phenotypic selection, Methods Enzymol. 154 (1987) 367–382.
[16] S. Morita, T. Kojima, T. Kitamura, Plat-E: an efficient and stable system for transient packaging of retroviruses, Gene Ther. 7 (2000) 1063–1066.

[17] J. Wienands, F. Freuler, G. Baumann, Tyrosine-phosphorylated forms of Ig beta, CD22, TCR zeta and HOSS are major ligands for tandem SH2 domains of Syk, Int. Immunol. 7 (1995) 1701–1708.
[18] T. Adachi, H. Flaswinkel, H. Yakura, M. Reth, T. Tsubata, The B cell surface protein CD72 recruits the tyrosine phosphatase SHP-1 upon tyrosine phosphorylation, J. Immunol. 160 (1998) 4662–4665.
[19] J. Wienands, O. Larbolette, M. Reth, Evidence for a preformed transducer complex organized by the B cell antigen receptor, Proc. Natl. Acad. Sci. USA 93 (1996) 7865–7870.
[20] Y. Hokazono, T. Adachi, M. Wabl, N. Tada, T. Amagasa, T. Tsubata, Inhibitory coreceptors activated by antigens but not by anti-Ig heavy chain antibodies install requirement of costimulation through CD40 for survival and proliferation of B cells, J. Immunol. 171 (2003) 1835–1843.
[21] J. Wienands, J. Schweikert, B. Wollscheid, H. Jumaa, P.J. Nielsen, M. Reth, SLP-65: a new signaling component in B lymphocytes which requires expression of the antigen receptor for phosphorylation, J. Exp. Med. 188 (1998) 791–795.
[22] T. Taniguchi, T. Kobayashi, J. Kondo, K. Takahashi, H. Nakamura, J. Suzuki, K. Nagai, T. Yamada, S. Nakamura, H. Yamamura, Molecular cloning of a porcine gene syk that encodes a 72-kDa protein-tyrosine kinase showing high susceptibility to proteolysis, J. Biol. Chem. 266 (1991) 15790–15796.
[23] B. Muller, L. Cooper, C. Terhorst, Molecular cloning of the human homologue to the pig protein-tyrosine kinase syk, Immunogenetics 39 (1994) 359–362.
[24] M. Fluck, G. Zurcher, A.C. Andres, A. Ziemiecki, Molecular characterization of the murine syk protein tyrosine kinase cDNA, transcripts and protein, Biochem. Biophys. Res. Commun. 213 (1995) 273–281.
[25] L.K. Gauen, Y. Zhu, F. Letourneur, Q. Hu, J.B. Bolen, L.A. Matis, R.D. Klausner, A.S. Shaw, Interactions of p59fyn and ZAP-70 with T-cell receptor activation motifs: defining the nature of a signalling motif, Mol. Cell. Biol. 14 (1994) 3729–3741.
[26] C. Fu, C.W. Turck, T. Kurosaki, A.C. Chan, BLNK: a central linker protein in B cell activation, Immunity 9 (1998) 93–103.
[27] K. Hayashi, R. Nittono, N. Okamoto, S. Tsuji, Y. Hara, R. Goitsuka, D. Kitamura, The B cell-restricted adaptor BASH is required for normal development and antigen receptor-mediated activation of B cells, Proc. Natl. Acad. Sci. USA 97 (2000) 2755–2760.
[28] M.T. Furlong, A.M. Mahrenholz, K.H. Kim, C.L. Ashendel, M.L. Harrison, R.L. Geahlen, Identification of the major sites of autophosphorylation of the murine protein-tyrosine kinase Syk, Biochm. Biophys. Acta 1355 (1997) 177–190.
[29] K.M. Kim, G. Alber, P. Weiser, M. Reth, Signalling function of the B-cell antigen receptors, Immunol. Rev. 132 (1993) 125–146.
[30] M. Iwashima, B.A. Irving, O.N. Van, A.C. Chan, A. Weiss, Sequential interactions of the TCR with two distinct cytoplasmic tyrosine kinases, Science 263 (1994) 1136–1139.
[31] V. Rolli, M. Gallwitz, T. Wossning, A. Flemming, W.W. Schamel, C. Zurn, M. Reth, Amplification of B cell antigen receptor signaling by a Syk/ITAM positive feedback loop, Mol. Cell. 10 (2002) 1057–1069.
[32] T. Wossning, M. Reth, B cell antigen receptor assembly and Syk activation in the S2 cell reconstitution system, Immunol. Lett. 92 (2004) 67–73.