FEBS Letters 350 (1994) 263–265

FEBS 14396

Tec homology (TH) adjacent to the PH domain

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Received 8 July 1994

Abstract

The pleckstrin homology (PH) domain is extended in the Btk kinase family by a region designated the TH (Tec homology) domain, which consists of about 80 residues preceding the SH3 domain. The TH domain contains a conserved 27 amino acid stretch designated the Btk motif and a proline-rich region. Sequence similarity was found to a putative Ras GTPase activating protein and a human interferon-γ binding protein both in the PH domain and the Btk motif region. SLK1/SSP31 protein kinase and a non-catalytic p85 subunit of PI-3 kinase had similarity only with the proline rich region. The identification of a PH domain extension in some signal transduction proteins in different species suggests that this region is involved in protein–protein interactions.

Key words: Btk; Bruton's agammaglobulinemia tyrosine kinase; Signal transduction; Cytoplasmic tyrosine kinase; XLA, X-Linked agammaglobulinemia

1. Introduction

Kinases in general contain several domains each of which has a distinct function [1,2]. Many signal transduction proteins, including protein tyrosine kinases (PTKs), protein serine/threonine kinases, Ras GTPase activating proteins (GAPs) and phospholipases, have a region related to pleckstrin, called the pleckstrin homology (PH) domain [3–6]. Although the function of the PH domain is mainly unknown, a point mutation affecting a conserved arginine residue in the PH domain of the cytoplasmic PTK Btk causes the human disease X-linked agammaglobulinemia [7] and X-linked immunodeficiency in mice [8,9].

Btk [10,11] (entry hsatk) forms a family with two other PTKs, Itk/Tsk [12,13] (mmtyrkin) and Tec [14] (ju0215) in which the PH domain is followed by an SH3 (Src homology 3) domain. The conserved stretch of approximately 80 residues between the two regions has tentatively been designated the TH (Tec homology) domain [15]. In the N-terminus of the TH domain is a highly conserved pattern of 27 amino acids, here called the Btk motif, which is followed by a proline-rich region (PRR). Btk family TH sequences were compared to relevant databases to search for related sequences.

2. Materials and methods

The sequence databases GenBank, EMBL and PIR were analyzed with the programs FASTA, TFASTA [16], Profilesearch [17] and Tprofilesearch as implemented in the GCG [18] and EGCG (available from EMBL) program packages. The multiple sequence alignment was obtained with the GCG software.

3. Results and discussion

The TH domain consists of a conserved Btk motif and PRR, both of which appear in the other members of the Btk family. In the search the Btk motif was found to follow the PH domain in a putative GAP protein from *Drosophila melanogaster* [19] (dmgtpaap) and in a human interferon-γ binding protein (IGBP) [20] (a09787) (Fig. 1A). The similarity was restricted to the PH domain and the Btk motif. In contrast to the Btk family the PH domain of Ras GAP is not located in the N-terminus and both Ras GAP and the partial IGBP sequence available lack apparent kinase (SH1), SH2 and SH3 domains (Fig. 1B). Similarity was limited to the PRR in the *Saccharomyces cerevisiae* protein kinase SLK1/SSP31 [21,22] (jq1432) and the non-catalytic p85 subunit of PI-3 kinase [23–25] (p85a_human). Both these proteins lack a PH domain.

The alignment of the sequences in Fig. 1. indicates that conservation of both the PH and the whole TH domain (Btk motif followed by PRR) is typical for Btk family members. The Btk motif is highly conserved also in the IGBP and Ras GAP sequences. There are no gaps in any of the sequences in this region. Of the 27 residues in the Btk motif 7 are invariant and 10 are conserved. The region contains a conserved histidine and three cysteines,

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Abbreviations: Btk, Bruton's agammaglobulinemia tyrosine kinase; GAP, GTPase activating protein; IGBP, interferon-γ binding protein; PH, pleckstrin homology; PRR, proline rich region; PTK, protein tyrosine kinase; SH, Src homology; TH, Tec homology; XLA, X-linked agammaglobulinemia.

SSDI 0014-5793(94)00783-7

a **PH domain**

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                                             #
     IGBP  14 HVDSVSLLLF MIKRAQGRKR FGMKNFKKRW FRLTNHEFTY ........HK  55
  Ras GAP 769            MTKYPTSRKR FG.RQFKQRH FRLTTHSLSY ........AK 799
      Itk   1 MNNFILLEEQ LIKKSQQKRR TSPSNFKVRF FVLTKASLAY FE.....DRH  45
      Tec   1 MNFNTILEEI LIKRSQQKKK TSLLNYKERL CVLPKSVLSY YE.....GRA  45
      Btk   1  MAAVILESI FLKRSQQKKK TSPLNFKKRL FLLTVHKLSY YEYDFERGRR  49
Consensus     mn    Leei  iKrsqqkkr ts  nfK R  f Lt h lsY yE     gr

     IGBP  56 SKGDQPLYSI PIENILAVEK LEEE...... .......... ........SF  81
  Ras GAP 800 SKGKQPICDI PLQEIASVEQ LKDK...... .......... ........SF 825
      Itk  46 GKKRTLKGSI ELSRIKCVEI VKSDIS.... .......... .......IPC  74
      Tec  46 EKKYR.KGVI DISKIKCVEI VKNDDGV... .......... .......IPC  74
      Btk  50 GSK...KGSI DVEKITCVET VVPEKNPPPE RQIPRRGEES SEMEQISIIE  96
Consensus      kk qpkgsI     I cVEi vk                             I

                                             *
     IGBP  82 KMKNMFQVIQ PERALYIQAN NCVEAKDWID ILTKVSQCNQ            121
  Ras GAP 826 KMQNCFKIVH NDRSLIVQTT NCVEEREWFD LLHKICLMNS            865
      Itk  75 HYKYPFQVVH DNYLLYVFAP DCESRQRWVL TLKEETRNNNSL          116
      Tec  75 QNKFPFQVVH DANTLYIFAP SPQSRDRWVK KLKEEIKNNNNI          116
      Btk  97 RFPYPFQVVY DEGPLYVFSP TEELRKRWIH QLKNVIRYNSDL          138
Consensus       k pFqvvh d   Lyvfap  c  r rW    Lk     N
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TH domain

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     IGBP 122 LTVYHPSAYL SGHWLCCRAP SDSAPGCSPC TGGLPA                159
  Ras GAP 866 MQYFHPSAFV SGFYSCCGRS DENSPGCKKV LDKTMD                903
      Itk 117 VSKYHPNFWM DGRWRCCSQL EKPAVGCAPY DPSKNA..              152
      Tec 117 MIKYHPKFWA DGSYQCCRQT EKLAPGCEKY NLFESS..              152
      Btk 139 VQKYHPCFWI DGQYLCCSQT AKNAMGCQIL ENRNGSLK              176
Consensus       kyHP f w dG y CC  q  k apGC
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Btk motif

SH3

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      Itk 153 .......SKK PLPPTPED.. ...NRR.... ...SFQEPE ETLVI     177
      Tec 153 .......IRK TLPPAPEI.. ...KKRRPPP PIPPEEENT EEIVV     184
      Btk 177 PGSSHRKTKK PLPPTPEEDQ ILKKPLPPEP AAAPVSTSE LKKVV     220
Consensus            kK pLPPtpe       k r P P   p  e e
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Proline rich region

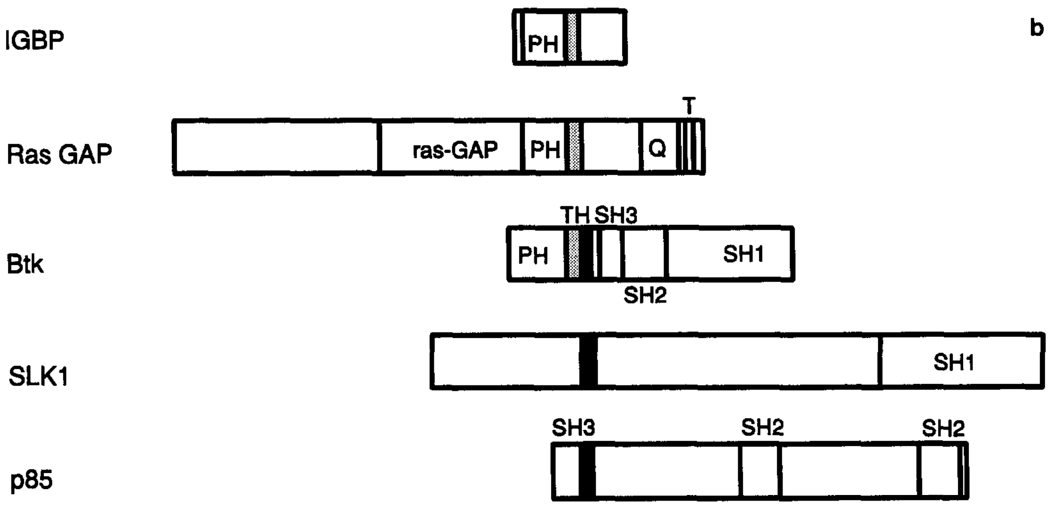

Fig. 1. (A) Alignment of proteins related to Btk family PH and TH domains. The TH domain contains two distinct motifs, the Btk motif and a proline rich region. The similarities in the PH domain were extended in IGBP and Ras GAP to the Btk motif of the TH domain. # indicates the conserved residue causing X-linked agammagobulinemia in man [7] and X-linked immunodeficiency in mice [8,9] when mutated. The invariant tryptophan in 45 PH domains [6] is indicated with *. The invariant residues are in capital letters and conserved in small letters in the consensus sequence. The underlining shows the location of the two motifs in the Btk family. (B) Domain structure of the proteins having similarity to TH domain. The structures are aligned according to PH and TH domains in Btk. The Btk motif is grey and the proline rich region is black. Btk represents all the Btk family members, since they all have similar domain structure. Q denotes the glutamine-rich region and T the threonine-rich region in Ras GAP.

residues that are involved in zinc fingers. However, the Btk motif sequence does not match with any of the known zinc finger topologies [26]. The pattern HXXXXXXXXXXCCXXXXXXXXXC was found in addition to the Btk family only from albumins and a virus env polyprotein. The pattern that contained all the invariant residues of the Btk motif was present only in Btk family members. Conservation of the Btk motif in these widely different proteins suggests an important function for the TH domain.

Furthermore, the PH domains of the proteins in the Fig. 1 are more related than PH regions in general. The only major difference is the large insertion in the Btk sequence in a region which is variable also in other PH sequences [6]. The five aligned sequences have 15 invariant residues compared to only one in the 45 PH domains studied previously. Random sampling of the 45 domains in ten groups of five indicated 1–9 invariant residues. To avoid bias from related Btk family members only one of them, Btk, was compared to the IGBP and Ras GAP, and 19 invariant residues were found. Random sampling of three unrelated PH domains indicated 3–13 invariant amino acids.

PRRs are commonly involved in protein-protein interactions [27,28]. When the Btk family members were aligned with SLK1/SSP31 residues 317–343 and the amino acids 84–97 of p85 subunit sequences a consensus sequence KXkKpLPPtpeXXXIXXkXXrPpp was obtained for the PRR. A short stretch in the PRR is duplicated in Btk possibly having a specific function. Only p85 has a similar duplicated stretch, although a gap is required for optimal alignment. The PRRs of the proteins studied here might be required for signal transduction. A peptide corresponding to the p85 PRR blocks activation of the Pl-3 kinase by preventing binding of the SH3 domain of the Src family PTKs Lyn and Fyn to the p85 subunit [29].

These results demonstrate that the PH domain is extended in some signaling molecules. These proteins contain a PH domain followed immediately by the Btk motif of the TH domain, but may differ considerably in other regions of the molecule, indicating that this PH domain extension may be of functional importance in various signaling pathways in different species. A complete TH domain, containing both the Btk and the PRR motif, has so far not been found in proteins outside the Btk family and may be a hallmark of these cytoplasmic PTKs.

Acknowledgements: This work was supported by the Axel and Margaret Ax:son Johnson Foundation, the Swedish Cancer Society, the Swedish Medical Research Council, and Sven and Dagmar Salén Foundation (to C.I.E.S) and the Swedish Natural Science Research Council (to L.N.).

References

[1] Hanks, S.K. (1991) Curr. Opin. Struct. Biol. 1, 369–383.

[2] Bolen, J.B. (1993) Oncogene 8, 2025–2031.

[3] Haslam, R.J., Koide, H.B. and Hemmings, B.A. (1993) Nature 363, 309–310.

[4] Mayer, B.J., Ren, R., Clark, K.L. and Baltimore, D. (1993) Cell 73, 629–630.

[5] Shaw, G. (1993) Biochem. Biophys. Res. Commun. 195, 1145–1151.

[6] Musacchio, A., Gibson, T., Rice, P., Thompson, J. and Saraste, M. (1993) Trends Biochem Sci. 18, 343–348.

[7] de Weers, M., Mensink, R.G.J., Kraakman, M.E.M., Schuurman, R.K.B. and Hendriks, R.W. (1994) Hum. Mol. Genet. 3, 161–166.

[8] Thomas, J.D., Sideras, P., Smith, C.I.E., Vorechovský, I., Chapman, V. and Paul, W.E. (1993) Science 261, 355–358.

[9] Rawlings, D.J., Saffran, D.C., Tsukada, S., Largaespada, D.A., Grimaldi, J.C., Cohen, L., Mohr, R.N., Bazan, J.F., Howard, M., Copeland, N.G., Jenkins, N.A. and Witte, O.N. (1993) Science 261, 358–361.

[10] Vetrie, D., Vorechovský, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammarström, L., Kinnon, C., Levinsky, R., Bobrow, M., Smith, C.I.E. and Bentley, D.R. (1993) Nature 361, 226–233.

[11] Tsukada, S., Saffran, D.C., Rawlings, D.J., Parolini, O., Allen, R.C., Klisak, I., Sparkes, R.S., Kubagawa, H., Mohandas, T., Quan, S., Belmont, J.W., Cooper, M.D., Conley, M.E. and Witte, O.N. (1993) Cell 72, 279–290.

[12] Siliciano, J.D., Morrow, T.A. and Desiderio, S.V. (1992) Proc. Natl. Acad. Sci. USA 89, 11194–11198.

[13] Heyeck, S.D. and Berg, L.J. (1993) Proc. Natl. Acad. Sci. USA 90, 669–673.

[14] Mano H., Mano, K., Tang, B., Koehler, M., Yi, T., Gilbert, D.J., Jenkins, N.A., Copeland, N.G. and Ihle, J.N. (1993) Oncogene 8, 417–424.

[15] Smith, C.I.E., Islam K.B., Vorechovský, I., Olerup, O., Wallin, E., Rabbani, H., Baskin, B. and Hammarström, L. (1994) Immunol. Rev. 138, 159–183.

[16] Lipman, D.J. and Pearson, W.R. (1985) Science 227, 1435–1441.

[17] Gribskov, M., McLachlan, A.D. and Eisenberg, D. (1987) Proc. Natl. Acad. Sci. USA 84, 4355–4358.

[18] Devereux, J., Haeberli, P., Smithies, O. (1984) Nucleic Acids Res. 12, 387–395.

[19] Gaul, U., Mardon, G. and Rubin, G.M. (1992) Cell 68, 1007–1019.

[20] Novick, D., Mory, Y., Fischer, D.G., Revel, M. and Rubinstein, M. Patent no. EP0369413-A2, 23 May 1990.

[21] Costigan, C., Gehrung, S. and Snyder, M. (1992) Mol. Cell. Biol. 12, 1162–1178.

[22] Irie, K., Araki, H. and Oshima, Y. (1991) Gene 108, 139–144.

[23] Escobedo, J.A., Navankasattusas, S., Kavanaugh, W.M., Milfay, D., Fried, V.A. and Williams, L.T. (1991) Cell 65, 75–82.

[24] Skolnik, E.Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A. and Schlessinger, J. (1991) Cell 65, 83–90.

[25] Otsu, M., Hiles, I., Gout, I., Fry, M.J., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A.D., Morgan, S.J., Courtneidge, S.A., Parker, P.J. and Waterfield, M.D. (1991) Cell 65, 91–104.

[26] Schwabe, J.W.R. and Klug, A. (1994) Nature Struct. Biol. 1, 345–349.

[27] Williamson, M.P. (1994) Biochem. J. 297, 249–260.

[28] Ren, R., Mayer, B.J., Cicchetti, P. and Baltimore, D. (1993) Science 259, 1157–1161.

[29] Pleiman, C.M., Hertz, W.M. and Cambier, J.C. (1994) Science 263, 1609–1612.