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BRUTON TYROSINE KINASE (BTK) IN X-LINKED AGAMMAGLOBULINEMIA (XLA)

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1. ABSTRACT

X-linked agammaglobulinemia (XLA) is a heritable immunodeficiency disorder that is caused by a differentiation block leading to almost complete absence of B lymphocytes and plasma cells. The affected protein is a cytoplasmic protein tyrosine kinase, Bruton's agammaglobulinemia tyrosine kinase (Btk). Btk along with Tec, Itk, Bmx and Txk belong to a distinct family of protein kinases. These proteins contain five regions; PH, TH, SH3, SH2 and kinase domains. Mutations causing XLA may affect any of these domains. About 380 unique mutations have been identified and are collected in a mutation database, BTKbase. Here, we describe the structure, function, and interactions of the affected signaling molecules in atomic detail.

2. INTRODUCTION

X-linked agammaglobulinemia (XLA) is a human immunodeficiency disorder, which is caused by a B lymphocyte differentiation arrest affecting the transition of B cell progenitors into mature B lymphocytes. The disease afflicts about 1/200,000 individuals (1). XLA is frequently recognized as the prototype of primary immunodeficiency (PID) (1)and was the first human immune disorder in which an underlying defect - the absence of gammaglobulins - was clearly identified (3). XLA is characterized by an increased susceptibility to infections, mainly those caused by extracellular bacteria (1, 3). In affected individuals, enteroviral infections frequently run a severe course and often resist therapy (1, 4, 5). The gene affected in XLA was found to encode a novel cytoplasmic tyrosine kinase designated Bruton's tyrosine kinase, Btk (6-8).

The increased susceptibility, mainly to bacterial infections in XLA, most often begins during the first year of life when the transferred maternal Ig has been catabolized. There is a pronounced decrease in Ig levels of all isotypes and a virtual absence of humoral response to recall antigens. B lymphocyte and plasma cell numbers are decreased, whereas T lymphocyte subsets are normal and may show a relative increase. The defect is caused by a differentiation arrest confined to the B cell lineage, distinguishing XLA from several other Ig deficiencies. B lineage cells in all organs are affected resulting in a reduced size of secondary lymphoid organs such as lymph nodes and tonsils.

3. SPECTRUM OF INFECTIONS AND TREATMENT

The onset of symptoms varies extensively; most patients will show an increased frequency of infections during their first year of life, whereas a few may be asymptomatic until adolescence. Pneumonia, otitis media, and diarrhea are frequent clinical presentations. Sinusitis, conjunctivitis, and pyoderma are also prevalent. Spread of the infection through the blood results in septicemia, meningitis, septic arthritis and sometimes osteomyelitis. Thus, a highly increased frequency of infections is seen essentially in all organs, with the possible exception of the urinary tract, in which only infections with mycoplasma species seem to be overrepresented (4, 9).

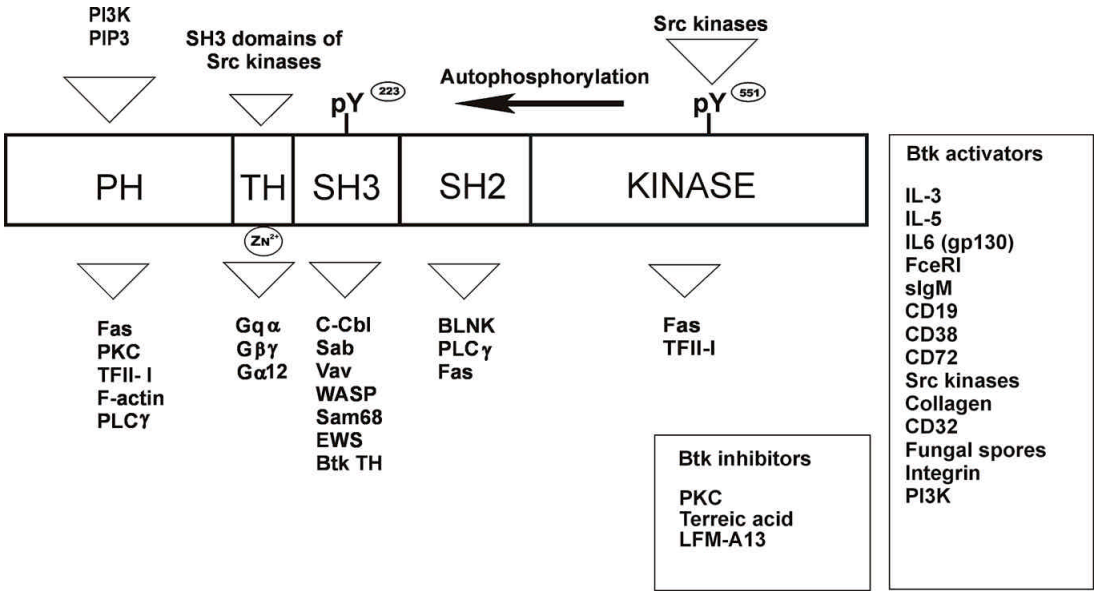

Figure 1. The binding partners of Btk and the molecules shown to regulate the Btk activity. The figure is a modification from a figure, represented in a reference article (41).

Typically in patients with XLA, the infections are bacterial and are caused by *Haemophilus influenzae* or *Streptococcus pneumoniae*. These infections affect all individuals with defective humoral immune responses. However, in contrast to most other primary humoral immunodeficiencies, enteroviral infections may cause an often fatal, slowly progressing disease affecting the central nervous system (5, 9). Thus, antibodies directed against these viruses play a pivotal role in the immune defense.

Bacterial infections are treated with a high dose of antibiotics for prolonged periods. The enteroviral infections may respond to gammaglobulins, but this is not always the case. However, it seems as if high dose gammaglobulin prophylaxis frequently prevents enteroviral infections (9). High dose gammaglobulin given by intravenous or subcutaneous infusions also decrease the number of bacterial infections.

4. THE XLA GENE ENCODES A TYROSINE KINASE

The gene defective in XLA was mapped to the Xq21.3-22 region in the mid-portion of the long arm of the X-chromosome (10-13). *BTK* encodes a cytoplasmic protein-tyrosine kinase (PTK). Btk forms a distinct family together with Tec, Itk and Bmx (Table 1). Txk (21, 26) also belongs to this family. These proteins are called the Tec family, as Tec was the first kinase of this family to be isolated.

Tec family members have in the N-terminus pleckstrin homology (PH) domain which has membrane-localizing function. The PH domain is followed by the TH region, which is unique to the Tec family. The Src homology 2 (SH2) and SH3 domains have binding functions, whereas the kinase domain is catalytic and phosphorylates tyrosine residues.

5. REGULATION OF BTK ACTIVITY AND ITS CONNECTION TO SIGNAL TRANSDUCTION PATHWAYS

The Tec family protein appears to be involved in a vast array of signal transduction pathways. The signaling impairment in Xid mice suggest a pivotal role for Btk in lympho-hematopoietic growth and differentiation (28-33). The characteristics of the Tec family of PTKs are summarized in Table 1. Except for Txk, which contains an unique cysteine string at the N-terminus, the members of the Tec family share the same organization consisting of PH, TH, SH3, SH2, and SH1 domains. Recently, new members of the family have been found from other vertebrates - homologues from skate and zebra fish (Itk) have been reported (19, 25). The multiplicity of signals is guaranteed by different specificities and interplay of the domains of various proteins. A critical role for these domains in Btk associated signal transduction has been demonstrated by mutations found in XLA patients (34-37).

The crucial role of Btk in B cell differentiation has been studied by searching molecules regulating the activity of Btk and connecting it to various signal transduction pathways (38-41). To date, the main pathways Btk have been shown to participate are the B-cell antigen receptor (BCR), the high affinity IgE receptor (Fc_eRI) in mast cells (42), IL-3 (43), IL-5 (44, 45) and IL-6 receptors (46), G-protein coupled receptors via association with Ga12, Gqa or ß? subunits (47-51) and the CD32, collagen or thrombin receptors in platelets (52-55). In Figure 1, the known interactions of Btk and the regulators of Btk activity in signaling pathways are summarized.

Table 1. A summary of the Tec family PTK characteristics. Alternative or previously used names are in parenthesisTec

Tec family member	Origin of abbreviation	Cell distribution / Expression pattern	Species/ Isoforms /Splice variants	Chromosomal location	Protein size (aa)	MW (kDa	Gene Bank accession no.	Ref
Btk (Atk, Bpk, Emb	Bruton's tyrosine kinase	Hematopoietic cells, not in T or plasma cells Bone marrow, spleen, monocytes, lung, pancreas	Human Mouse	Xq22 Syntetic Xq22	659 659	76 76	X58957 L29788	6, 7 14
Tec	Tyrosine kinase expressed in hepatocellular carcinoma	T lymphocytes and myeloid cells Ubiquitously expressed	Human Tec Mouse[1] TecIIa TecIIb TecIII TecA/Tec IV[2]	4p12 5 5 5 5	631 602 624 608 630	74 70 72 62 74	D29767 AF071946 AF071946 AF071936 AF071936	15 16 16 16 16
Itk (Tsk, Emt	IL-2 inducible T-cell kinase	T lymphocytes and mast cells Thymus, kidney in ZF	Human Mouse Zebrafish	5q34 15	620 625 616	72 72 72	D13720 L00619 AF016326	17 18 19
Bmx (Etk	Bone marrow kinase gene on the X chromosome	Bone marrow, endothelial cells Fetal and adult heart, lung, prostate	Human Mouse	Xp22.2 X	675 651	78 75	X83107 U88091	20
Txk (Rlk	Tec-related protein tyrosine kinase	T lymphocytes and myeloid cells, Thymus	Human[3] Mouse[3]	4p12 5	527 and 502 527 and 473	61 and 58[4] 61 and 55[4]	L27071 U16145	21 22
Btk29A[5]	D. melanogaster Btk family kinase at 29A	In many various parts of a developing fruit fly[6]	Drosophila Type 1 melano-gaster Isoform p55 Type 2 Isoform p66	2-[24][7]	603 786 465 590	67 87 53 66	AB009840 AB009841 M16599 M16599	23 23 24 24
Skate PTK	Raja eglanteria	Spleen			628	72	U85659	25

[1]Only the main splice variants are shown [2]*TecIV* is identical with *TecA* except for five aa changes (16. [3]Both human *TXK* and mouse Txk have two splice variant arising by alternative initiation of translation [4]According to experimental data the human and mouse protein products migrated as 58- and 55-kDa and 58- and 52-kDa proteins, respectively (27. [5]Also known as src28C, Tec29, Src29A, dsrc29A[6]Adult (dorsal vessel, cardia and ejaculatory bulb, embryo (amnioserosa, anterior midgut primordium, ectoderm, embryonic central nervous system etc larva (gonad, imaginal disc, larval central nervous system, lymph gland, central nervous system ovary (nurse cell, oocyte, ovary and ring canal and prepupa and pupa (genital disc, imaginal disc, histoblast, lamellocyte, pupal/adult digestive system. [7]Cytogenetically located at 29A1-2

In B-cells cross-linking of BCR activates Btk (56), through a phoshorylation cascade. First, after BCR cross-linking, the generation of PtdIns(3,4,5)P_3 by phosphatidylinositol 3-kinase (PI3K) leads to PH domain-mediated Btk translocation to the membrane (57). Next, the highly conserved activation loop tyrosine, Y551, is phosphorylated by a transient association of Btk with Src kinases causing a marked increase in Btk activity. This is followed by Btk autophosphorylation at the second site, SH3 domain Y223, inducing the full activation of Btk (58-60). The stimulation of BCR is intimately linked to the activation of three cytoplasmic tyrosine kinase families, namely the Src family, the Tec family and the Syk family (38). Time course -studies implicate temporal activation of these proteins. Src family kinases are activated first (5-10 seconds). This is followed by activation of Btk (2-5 minutes) and Syk family of kinases (10-60 minutes) (61). This indicates a downstream role for Btk and Syk kinases in the signaling pathway which is initiated by the Src kinases. Btk activation has been shown to correlate with the dose of Src family kinase activity (60).

Increasing evidence suggests that Btk is a critical component in BCR–coupled calcium signaling pathway (62-64). A chicken DT40 B cell Btk knock-out and mutational analysis of Btk indicated that both the PH and the SH2 domain are essential for the PLC-?2 activation (62, 64), another critical component of calcium signaling. The function of the PH domain of Btk has been explained by its ability to bind Ptd Ins(3,4,5)P_3 (65-66), which leads to the translocation of Btk to the cell membrane and to the subsequent activation by the Src family kinases (57, 58), see above. A newly identified B-cell linker protein (BLNK; SLP-65) as one of the major Btk SH2 binding proteins could explain the role of Btk SH2 domain in calcium signaling (67). Once phosphorylated by Syk, BLNK is thought to act as a scaffolding molecule assembling the downstream targets of antigen activation. In this case, Btk SH2 domain and PLC-?2 are both suggested to bind to the BLNK docking sites, thereby bringing Btk into close proximity with PLC-?2 (67, 68). The activation of PLC-?2 generates the formation of Ins(1,4,5)P_3 and thereby induces intracellular calcium release, extracellular calcium influx and PKC activation (62-64, 69). Further, Btk has been demonstrated to interact with PKC isoforms resulting in inhibition of Btk (70-71), which might contribute to a regulating role for PKC in calcium signaling.

Recently, Btk PH domain has been reported to bind F-actin (72) and cytoskeletal regulation, mediated by small GTPases, has been demonstrated for Btk (73). The PH-TH and kinase domains of Btk have also been shown to be responsible for the regulation of nuclear localization and transcriptional activity of TFII-I (BAP-135), a multifunctional transcription factor, suggesting a novel pathway for Btk (74, 75). The activation of another nuclear factor, NF-κB, is found to be a downstream target of Btk in response to BCR engagement (76, 77). NF-κB has also been implicated in the up-regulation of Bcl-x (78) and this, together with the observation that Btk acts as an anti-apoptotic protein upstream Bcl-x (79), might contribute to the B-cell deficiences XLA and xid. In fact, Btk has been identified to act as a dual-function regulator of apoptosis promoting radiation-induced apoptosis, but inhibiting Fas-activated apoptosis in chicken DT-40 lymphoma B-cells (80, 81).

A variety of molecules have been reported to bind Btk SH3 domain: Wiskott-Aldrich syndrome protein (WASP), Ewing's sarcoma protein (EWS), Sam68 (Src-associated in mitosis 68 kD), Sab (SH3 domain-binding protein which preferentially associates with Btk), Cbl and Vav (82-85). Moreover, Btk SH3 domain has been shown to bind to its own proline-rich peptide of the TH domain thus possibly regulating its own catalytic activity (87).

The search for the inhibitors of Btk could be advantageous for the probing into the functions of Btk in immune cell systems or for the therapeutic applications. As terreic acid, a quinone epoxide antibiotic, has been identified to be a specific inhibitor between PKC and Btk PH domain in mast and other immune cells, it might serve as a useful tool for understanding roles of Btk in diseases (87). By using a docking procedure a chemosensitizing anti-Btk agent, a-cyano- ß-hydroxy- ß-methyl-N-(2,5-dibromophenyl)-propenamide (LMF-A13), has been constructed. LMF-A13 increased the chemosensitivity of Btk-positive B-lineage leukemia cells to vincristine and ceramide, the inducers of apoptosis (88).

6. STRUCTURAL CONSEQUENCES OF Btk MUTATIONS

The structure of two and the partial structure of another of the five Btk domains (PH domain plus Btk motif of TH domain and SH3 domain) have been determined (89-91) and the others have been modeled to study structure-function and genotype-phenotype interactions (92-95). The gene defect leading to XLA has been characterized in a large number of patients. The mutations have been collected into a database called BTKbase (34-37). The BTKbase is available at World Wide Web at http://www.uta.fi/imt/bioinfo/btkbase. BTKbase and our other immunodeficiency mutation databases are maintained with MUTbase program suite (96). Patents are identified with a specific Patent Identification Number (PIN) (97).

Analysis of the registry indicated that in the 636 XLA patients, from 556 unrelated families mutations are scattered throughout the entire length of *BTK* gene. The proportion of unique mutations is 63% (401 cases), and the distribution of the mutations in the five structural domains essentially corresponds to the length of the domains. Exonic mutations are distributed as follows: 212 families had missense mutations, 103 had nonsense mutations, 35 showed insertions, and 100 had deletions. In addition, there are 86 intron mutations affecting splice sites. The gene defect of 15 gross deletions have not been characterized in detail. As expected, the missense mutations appear mainly in the first two positions within the codon. Eight of 18 CpG containing arginine residues were affected, whereas none of the residual 15 CpG sites encoding nonarginine residues were mutated. CpG dinucleotides are involved in all the

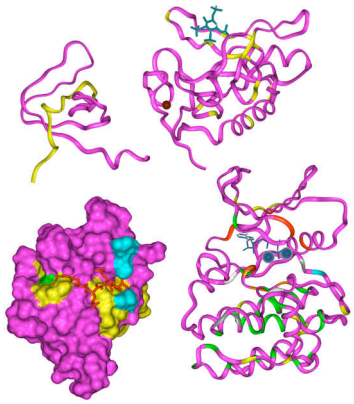

Figure 2. Three-dimensional structures of Btk domains. Top left, Btk SH3 domain. The inframe deletion of the C-terminus of the domain is shown in yellow. Top right, Btk PH domain. The locations of the XLA-causing missense mutations (yellow) are concentrated mainly on the phoshphatidyl inositol (green) binding site region. Zn^{2+} is in cyan. Bottom left, Btk SH2 domain model. The disease-causing mutations are in yellow. Ligand peptide is in blue. Bottom right, Btk kinase domain model with color coded consequences of missense mutations. The putative structural alterations are in green, ATP binding site mutatations in red, catalytic residues in gold, substrate binding site mutations in white, thephosphotyrosine bidning site in cyan, and domain-domain or protein-protein interaction sites in yellow. ATP and Mg^{2+} ions are in cyan.

cases where a high number of unrelated families have the same mutation except for the initiation site. The larger deletions encompass whole exons. The structures and models of the domains have been used to describe the putative structural consequences of each of the XLA mutations.

7. PH DOMAIN

Highly divergent pleckstrin homology (PH) domains of about 120 residues have been found in a number of signaling and cytoskeletal proteins including protein tyrosine and serine/threonine kinases and their substrates, phospholipase C, GTPase activating proteins, guanine nucleotide releasing factors, and adaptor proteins (98-104). The Tec family kinases are the only PTKs which contain a PH domain. The three dimensional structure has been determined for several PH domains including Btk (90, 91). Despite very low sequence identity, the structures have the same fold consisting of a ß-barrel formed of two ß-sheets and a C-terminal α-helix that caps one end of the ß -barrel.

The function of the PH domain is still somewhat unclear, but in many cases it acts as a membrane localisation domain. The N-terminal half of certain PH domains binds inositol compounds thereby being possibly important for the membrane localisation for proteins that have to be at least transiently close to membrane (39, 105-108). Many PH domains have been shown to interact with subunits of heterotrimeric G proteins (47-50, 109-111). Certain PH domains can bind also to protein kinase C (PKC) isoforms (112-115).

The structure of Btk PH domain mutant R28C has been determined (90) as well as the wild type and E41K mutants with Ins(1,3,4,5)P_4 (91). The inositol compound binds between loops ß1-ß2 and ß3-ß4 (91). E41K mutant was found to bind also another Ins(1,3,4,5)P_4 molecule. The affinities were measured to be in the order of 40 nM (91). Substitution of Btk PH domain residue, E41, by lysine was shown to increase phosphorylation of tyrosine residues and membrane targeting (105). Thus, the Btk phosphorylation might be linked to membrane interaction.

The residues interacting with phosphoinositides have been identified in Btk (91), PLC d1 (108), and ß-spectrin (107, 116) structures. PH domains are clearly electrostatically polarized. The positively charged surface of the PH domain binds to the negatively charged phosphate groups of the inositol molecules, which can play a role in membrane recruitment. The Ins(1,3,4,5) is bound to the Btk PH domain by residues K12, Q15, Q16, K17, K18, S21, N24, K26, R28, and Y39 (91). These residues are invariant in the Tec family except for K18, which is replaced by arginine in Itk.

Btk and Itk PH domains have been shown to interact with different PKC isoforms *in vitro* (112). PKC, a protein serine/threonine kinase, phosphorylates Btk and downregulates its activity (112). The minimal binding site in Btk PH domain contains also some inositol compound binding residues and PKC binding competes with PtdIns(4,5)P2 binding, suggesting that the binding sites overlap or are closely located(113).

Many receptors transmit signals via G proteins. Both a and ß? subunits of G proteins interact with PH domains. Btk (48, 110, 111) and Itk (110) are binding and they are activated by ß? subunits of G proteins. In addition Gqa (49) and Ga12 (50) subunits bind to and activate Btk. Ga (51) and ß? subunits (110, 111) are binding to the C-terminus of the PH domain and to the Btk motif of the TH domain (residues 108-162).

Most of the Btk PH domain mutations are concentrated in the binding site region where they could disturb interactions (Figure 2). The electrostatic properties of the domain change due to many of the missense mutations. The structure of the Btk PH domain with the bound Ins(1,3,4,5)P_4 is in Fig X with the locations of XLA-causing missense mutations. The mutation and binding sites in the Tec family members have been compared based on the Btk PH domain structure and the models of the other family members (117).

8. TH DOMAIN

The Tec family members have a TH domain of 60-80 amino acids (118-120) that contains two parts. N terminal Btk motif is followed by a proline rich region (PRR) (119, 120). The Btk motif contains HC_3 pattern (one histidine and three cysteines) which binds stabilising Zn^{2+} ion (90, 120).

The proline rich region in Btk binds to certain SH3 domains (121-123), possibly also intramolecularly to the adjacent SH3 domain (Okoh and Vihinen, submitted) as in Itk (124), thereby regulating the function of these kinases. The whole TH domain can be found only in the Tec family members. Btk motif follows PH domain also in several forms of Ras GTPase-activating protein 1 (Ras-GAP1) and in a putative interferon ?-binding protein (119). The function of the TH domain is unclear, although Src family SH3 domains bind to PRR *in vitro* and the Btk motif seems to be essential for interactions with G proteins.

The structure of the Btk motif has been determined together with the PH domain (90, 91). The motif has a novel fold coordinating Zn^{2+} (120) and it packs against the ß-sheet of the PH domain (90) (Figure 2). Three XLA-causing mutations have been identified in the Btk TH domain (C154S, C155G and C155R), in the conserved Zn^{2+} binding cysteines (120) thus destroying the binding site and affecting both folding and stability. The three dimensional structure indicates that the cysteines are crucial for metal binding and a substitution in any of them would affect folding.

The two 10 amino acid motifs in the PRR of Btk have been shown to interact with the SH3 domains of Fyn, Lyn and Hck (121 - 123). The binding site was localized to the first PRR repeat in the TH domain (121, 123).

Site-directed mutations of the polyproline II (PPII) helix forming proline residues in the PRRs of Btk abolish binding to SH3 domain (121, 123). Mutations, P189A and P192A (121, 123), are likely to alter the conformation such that the polyproline stretch can no longer be recognized. Also, mutation in the conserved polyproline binding region of Fyn (W119L) abolished the binding (123). Erythropoietin and IL3 stimulation induces the specific binding of Vav to Tec through the TH domain (125). An unidentified, 72 kDa protein, binds to residues 186-192 in the TH domain of Btk (123).

A mutation has been found from the latter PRR, E205D, but this patient has also a deletion leading to truncation in the PH domain at residue (126), thus it is not clear if the missense mutation could alone cause the disease phenotype. Another mutation replaces T184 in the beginning of the first PRR by proline. Also in this case there is a frameshift mutation truncating major part of the protein (127).

9. SH3 DOMAIN

SH3 domains bind polyproline stretches containing polypeptides and proteins. We have determined the solution structure of the Btk SH3 domain using two- and three-dimensional nuclear magnetic resonance (NMR) spectroscopy (92). The Btk has the typical SH3 domain topology of two short anti-parallel ß-sheets packed almost perpendicular to each other in a sandwich-like fold. Thermal unfolding of Tec family SH3 domains have been studied with CD spectroscopy and isothermal titration calorimetry (128).

Btk is activated by phosphorylation. Full activity is achieved only when the kinase domain activation loop tyrosine, Y551, and tyrosine Y223 in the SH3 domain are phosphorylated (58-60). Btk SH3 domain interacts with a number of ligands (Fig 1). After B cell receptor or T cell receptor stimulation the c-Cbl is rapidly phosphorylated (82). Phosphorylation of Y223 in the Btk SH3 domain prevents binding to WASP but does not affect intreraction with c-Cbl (129). In addition, the affinity to Syk is remarkably increased.

The NMR structure of the Itk SH3 domain with the TH domain PRR indicated intramolecular interaction, which could be important for the regulation of at least Itk function (124). Neither of the peptides for PRR sequences bound to Btk in *in vitro* filter binding assay (121). Btk was not shown to compete for the Fyn SH3 domain binding to the proline rich region (123), but, Btk SH3 domain binds very tightly to the peptide for N-terminal PRR *in vitro* (87). Recently we have shown that the N-terminal half of the Btk PRR binds specifically to Btk SH3 domain (Okoh and Vihinen, submitted). Docking the peptide indicated extensive hydrogen bonding and hydrophobic interactions with the SH3 domain and the feasibility of the intramolecular interaction. TH and SH3 domains might increase the substrate specificity of otherwise promiscuous kinase domain by binding simultaneously to different parts of the substrate protein, possibly together with the SH2 domain.

There are several nonsense mutations in the Btk SH3 domain, but no missense mutations have been found. Aberrant splicing and skipping of exon 9 leads to an in-frame deletion of 21 residues containing the 14 C-terminal residues of the SH3 domain in two unrelated families (93, 130) (Figure 2). Even though this protein is stable and it has full kinase activity *in vitro*, the patients have classical XLA. Deletion of the last three β-strands seems to distort the structure. According to molecular dynamics simulation, the spacing between the termini in the mutant protein corresponds to the normal Btk SH3 domain thus facilitating connection to the rest of the Btk without major changes in the overall scaffolding (93).

10. SH2 DOMAIN

SH2 domains of about 100 residues bind phosphotyrosine (pY) containing peptides and proteins. The specificity is gained by recognizing residues C-terminal to pY. SH2 domains play a central role in a number of cellular processes, including growth, immune response, metabolism, mitogenesis, motilily and gene transcription. SH2 domain-containing proteins take part into the coordination of signal transduction pathways. Binding specificities of several SH2 domains have been determined by using phoshotyrosine peptide libraries and phage display (131 - 133). Most of the XLA-causing Btk SH2 domain mutations disrupt the pY peptide binding sites (34-37).

Several high-resolution three-dimensional structures of SH2 domains have been determined. The

conserved fold contains a large central antiparallel ß-sheet and an associated smaller ß-sheet, flanked by a-helices on either side. The pY-containing ligand binds to the SH2 domain in an extended conformation (Figure 2). Crystal structures of SH2 domains with high-affinity peptides have shown that several residues are involved in phosphotyrosyl peptide binding. The number of residues binding in addition to pY varies from three to at least six of seven. However, also regions outside the actual pY binding region can be essential for affinity and specifity. The most important conserved interaction between the SH2 domain and its ligand is the direct interaction between the pY phosphate group and the guanidinium group of an arginine (R307 in Btk). Other important residues for the interaction are R288 and S309. The ligand binding specifity arises mainly from the nature of the pY+1 and pY+3 binding pockets.

In addition to Btk, disease-causing SH2 domain mutations are rare. A number of mutations are known in SH2D1A an adaptor protein that contains only an SH domain and short flanking regions. The consequences of the mutations have been discussed based on the model of the SH2 domain and compared to those in Btk (134). Mutations in the C-terminal SH2 domain of GAP have been found cause basal carcinomas (135) and a single nucleotide insertion in the Syk N-terminal SH2 domain leads lack of expression (136).

To date, 20 XLA-causing amino acid substitutions have been identified in the Btk SH2 domain (Figure 2). We have analysed the structural and functional properties of six Btk SH2 missense mutations (G302E, R307G, Y334S, L358F, Y361C and H362Q) (137). The binding of XLA mutation-containing SH2 domains to pY-Sepharose was reduced, varying between 1-13% of that for the native SH2 domain (137). The solubility of all the mutated SH2 domains was remarkably reduced, although mutated full-length Btk seemed to be less affected.

11. KINASE DOMAIN

The kinase domain of about 250 residues is the only catalytic part in most kinases including the Tec family PTKs. The three dimensional structure has been solved for several protein kinases. They all have the same scaffolding consisting of two lobes. The smaller N-terminal lobe contains five stranded antiparallel ß-sheet and one or two a-helices. The lower, C-terminal lobe has usually seven helices and some short ß-strands. ATP is bound in a cleft between the two lobes and substrate interacts mainly with the lower lobe (138).

Protein kinases are generally regulated by phosphorylation in the activation loop. When the enzyme is activated, the upper lobe rotates to lock the ATP molecule between the two domains. The ATP binding residues are the most conserved sites in all protein kinases suggesting that both PSKs and PTKs have the same direct in-line reaction mechanism (139, 140).

Btk kinase domain was originally modeled based on the cAPK structure (70) and subsequently based on the IRK and FGF. The models have been used to study the functional implications of XLA causing mutations (34-37, 93).

Almost half of the XLA-causing mutations appear in the kinase domain (37) which forms more than 40% of Btk. The mutations are mainly on one face of the kinase domain which is in charge of the ATP, Mg^{2+} and substrate binding. The hallmark residues provided basis for the sequence alignment. The upper domain is most conserved, which is in agreement with the conserved ATP binding mode. The major changes are in the loops connecting secondary structures and mainly on surface.

The mutations are markedly underrepresented in the upper lobe, which forms about one third of the domain's length (Figure 2). Putative structural description has been given for each XLA mutation (34-37, 93). There are several different types of missense mutations affecting structural, functional and interacting residues. The Btk kinase domain models in Figure 2 indicate the distribution of the mutations along the polypeptide chain. The severe XLA mutations are mainly in the ATP-binding cleft, the putative substrate binding region or in other functionally or structurally crucial sites. Milder XLA causing mutations are usually further away from the functional regions, with some exceptions.

12. ACKNOWLEDGEMENT

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