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Review

Syk and pTyr'd: Signaling through the B cell antigen receptor

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ABSTRACT

The B cell receptor (BCR) transduces antigen binding into alterations in the activity of intracellular signaling pathways through its ability to recruit and activate the cytoplasmic protein-tyrosine kinase Syk. The recruitment of Syk to the receptor, its activation and its subsequent interactions with downstream effectors are all regulated by its phosphorylation on tyrosine. This review discusses our current understanding of how this phosphorylation regulates the activity of Syk and its participation in signaling through the BCR.

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1. Introduction

B cells are essential components of the adaptive immune response, producing antigen-specific antibodies for the targeting of foreign molecules and cells. Foreign particles are recognized by the B cell receptor (BCR) for antigen, which comprises a membrane-bound immunoglobulin that binds the antigen and an associated heterodimer of disulfide-linked Ig-α (CD79a) and Ig-β (CD79b) subunits that are required for transducing this binding into alterations in intracellular signaling pathways [1,2]. Although none of the components of the BCR complex has intrinsic enzymatic activity, their engagement leads to the enhanced phosphorylation of multiple intracellular proteins on tyrosine, which occurs through the recruitment and activation of cytoplasmic protein-tyrosine kinases [1,3–6]. Spleen tyrosine kinase (Syk) is a critical component of this signaling machinery. Studies in "knockout" mice and cell lines indicate that Syk is essential for most of the biochemical responses to BCR engagement [7–10].

Syk was originally identified at the protein level as a 40-kDa proteolytic fragment containing the catalytic domain that was isolated from bovine thymus based on its ability to phosphorylate a synthetic peptide substrate [11]. A kinase of the same size was isolated from porcine spleen [12]. Antibodies against this active fragment identified the full-length enzyme as a 72-kDa protein, a size confirmed when the cDNA was sequenced [13,14]. Syk has at its amino terminus a tandem pair of Src homology 2 (SH2) domains separated by a 60-amino acid linker (linker A) (Fig. 1). A C-terminal catalytic domain is connected to the tandem SH2 domains by a stretch of 106-amino acids (linker B) that contains multiple sites of phosphorylation [15,16]. A role for Syk in cellular signaling was first identified in B lymphocytes [17–19], but Syk is expressed in many cell types including most cells of the hematopoietic system, and at lower levels in some epithelial cells, fibroblasts, hepatocytes, vascular smooth muscle cells, endothelial cells and neuronal cells [20,21]. A variant of Syk, SykB, which lacks a 23 amino acid "linker insert" from the linker B region due to alternative splicing of exon 7, also is expressed in a variety of cell types [22–25]. The ζ-chain associated protein of 70 kDa (Zap-70) is a Syk homolog expressed in T cells and NK cells and is the only other member of the Syk-family of cytoplasmic protein-tyrosine kinases [26]. Zap-70, like SykB, lacks a linker insert region.

2. Activation of Syk in B lymphocytes by binding to the antigen receptor

2.1. Phosphorylation of tyrosines in the receptor ITAM

Signaling is initiated in B cells when BCR complexes become aggregated. Rapidly following aggregation, the cytoplasmic tails of Ig-α and Ig-β are phosphorylated on tyrosines located in a stretch of amino acids known as an immunoreceptor tyrosine-based activation motif (ITAM) [1,3,27–29]. ITAMs are found not only within these two components of the BCR complex, but also within subunits of many additional immune cell receptors – examples include the T-cell antigen receptor (TCR), the immunoglobulin receptors FcεRI, FcγRI, and FcγRIIa, and the DAP12 component of NK cell activating receptors and integrins – and in cytoplasmic proteins of the ERM family [30–36]. ITAMs have the consensus sequence $(D/E)X_2YX_2LX_{7-10}YX_2(L/I)$. For Ig-α, this sequence is ENLYEGLNLDDCSMYEDI. The phosphorylation of the two tyrosines within the ITAM leads to the physical recruitment of

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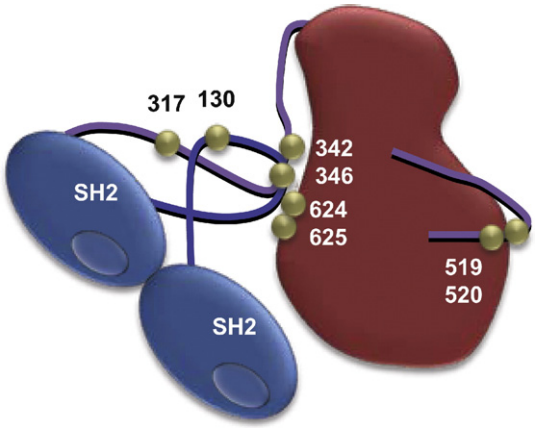

Fig. 1. Model of Syk showing approximate locations of major sites of tyrosine-phosphorylation.

Syk to the site of the clustered receptor in an interaction mediated by its tandem pair of SH2 domains [37–39]. Interestingly, a number of virally encoded proteins also contain ITAM sequences, which they use to co-opt Syk for their own nefarious purposes [35,40,41].

The initial phosphorylation of the ITAM is catalyzed by a member of the Src-family of cytoplasmic protein-tyrosine kinases. Several members of this family are expressed in B cells, but, of these, Lyn is thought to be particularly important and its elimination from mice yields a pronounced B cell phenotype [42–48]. Lyn associates with the clustered BCR [49–51] and catalyzes the phosphorylation of the first (N-terminal) tyrosine of the ITAM. This specificity can be demonstrated through in vitro analyses of kinase-substrate interactions and in a cellular model of BCR signaling reconstituted in insect cells [52,53]. Structural studies indicate that this specificity for the first ITAM tyrosine is dictated by the glutamate at the P-3 position and the glycine at the P+2 position [52]. The glutamate provides side chain hydrogen bonding interactions with residues in the catalytic cleft of Lyn while the glycine allows for the conformational flexibility needed for favorable binding of this region of the ITAM to the kinase. Accordingly, the stoichiometry of phosphorylation of the first tyrosine of Ig-α in activated B cells is much greater than that of the second [54].

Optimal signaling, however, requires the phosphorylation of both tyrosines of the ITAM as two phosphotyrosines are needed for a high affinity interaction with Syk's tandem pair of SH2 domains. The kinase that phosphorylates the second tyrosine of the ITAM is less certain, but a prime candidate is Syk, itself. Syk is capable of phosphorylating Ig-α in vitro and, in the reconstituted insect cell model system, its expression leads to the phosphorylation of both ITAM tyrosines even in the absence of Lyn [53]. Consistent with this observation, BCR-mediated signaling and the recruitment of Syk to the receptor can and does occur, albeit at a slower initial rate, in B cells that lack Lyn [7,44,55–57].

2.2. Activation of Syk by binding to phosphorylated ITAMs

Syk binds with high affinity to the doubly phosphorylated ITAM through its tandem pair of SH2 domains. These two domains are juxtaposed to form a Y-shaped structure that is held together and oriented properly through domain-domain interactions and through linker A for the simultaneous engagement of both ITAM phosphotyrosines. Linker A contains 3 α-helices, two of which form a coiled-coil [58]. Each SH2 domain recognizes one of the two pYXXL/I sequences within the phosphorylated ITAM via two major binding pockets, one that accommodates the phosphotyrosine and another that binds the hydrophobic side chain of the P+3 residue. Overall binding occurs in a head-to-tail orientation with the N-terminal SH2 domain of Syk interacting with the second phosphotyrosine of the ITAM and the C-terminal domain binding the first. The interaction surface between the two SH2 domains is sufficiently small and linker A sufficiently flexible to allow the tandem SH2 domains to exist in either an open or a closed conformation [58–61]. This facilitates Syk's interactions with ITAMs that vary in the degree of spacing between the two pYXXL/I binding motifs. Thus, Syk can bind with high affinity to immunoreceptors that have as few as 10 or as many as 15 residues separating the two ITAM phosphotyrosines [60].

Each of the SH2 domains contains all of the amino acid residues required to bind independently to one of the pYXXL/I sequences of the ITAM [58]. Thus, it has been suggested that Syk can interact also with proteins containing only one phosphotyrosine. The C-terminal SH2 domain, when expressed independently of the N-terminal domain and linker A, does, in fact, retain the ability to recognize and bind phosphopeptides on its own [62]. It is reasonable to speculate that the C-terminal SH2 domain mediates the initial recruitment of Syk to the BCR after phosphorylation of the first ITAM tyrosine by Lyn. Inactivation of the C-terminal SH2 domain does block the ability of Syk to signal when expressed in Syk-deficient DT40 B cells [37]. Subsequent phosphorylation of the second ITAM tyrosine by Syk would then allow for a much higher affinity interaction in which both SH2 domains are engaged. Interestingly, Syk also is recruited to and activated by engagement of a class of receptors exemplified by Dectin-1 and C-type lectin-like receptor 2 (CLEC-2) [36] that contain only a single pYXXL motif, suggesting an interaction mediated by a single SH2 domain. However, the mechanism by which Syk binds to these receptors is not completely understood as mutagenesis studies indicate that both SH2 domains are still required [63].

The binding of Syk to the phosphorylated ITAMs of clustered BCR complexes leads to its activation. In vitro studies indicate that the simple binding to Syk in solution of a dually phosphorylated peptide with the sequence of an ITAM is sufficient to fully activate the kinase [64–66]. In the X-ray crystal structure of the Syk homolog, Zap-70, the linker A region makes close contacts with the C-terminal lobe of the kinase domain and with residues in linker B forming what is referred to as a "linker-kinase sandwich" [67]. These interactions likely restrict the flexibility of the hinge region of the kinase domain preventing it from transitioning from an inactive to an active conformation. Engagement of the tandem SH2 domains by a dually phosphorylated ITAM reorients the domains and alters the conformation of linker A to disrupt these interactions. An analysis of Syk's structure by electron microscopy suggests an autoinhibited conformation comparable to that of Zap-70 indicating that Syk is likely to be regulated and activated in an analogous fashion [68]. Consequently, the binding of Syk to its reaction product, a dually phosphorylated ITAM, activates the enzyme and generates a positive feedback loop to promote the phosphorylation of additional ITAM tyrosines to recruit even more Syk molecules to the clustered BCR complexes [53,66].

Syk, recruited to the BCR, can catalyze the phosphorylation of ITAM tyrosines and at least one non-ITAM tyrosine (Y204) on Ig-α [69]. These residues lie within predicted AP-2 binding sites and the conversion of all three to non-hydrophobic residues severely impairs receptor internalization [69]. If the activity of Syk is then inhibited, the Syk-receptor complex rapidly dissociates and the receptor is internalized most likely due to its rapid dephosphorylation by protein-tyrosine phosphatases [70]. Thus, phosphorylated receptors that bind and activate Syk are retained at the cell surface while clustered, but nonphosphorylated receptors are internalized. The persistence of Syk-BCR complexes at the plasma membrane likely plays an important role in determining the length of time that Syk remains active following the initial engagement of the receptor. The prolonged activation of Syk is required for some receptor-stimulated events such as the activation of the NFAT transcription factor, which requires Syk to remain active for more than one hour following receptor ligation [70]. As we will see below, the binding of Syk to the adaptor protein BLNK/SLP-65 at the

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