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Structural Basis for the Inhibition of Tyrosine Kinase Activity of ZAP-70

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SUMMARY

ZAP-70, a cytoplasmic tyrosine kinase required for T cell antigen receptor signaling, is controlled by a regulatory segment that includes a tandem SH2 unit responsible for binding to immunoreceptor tyrosine-based activation motifs (ITAMs). The crystal structure of autoinhibited ZAP-70 reveals that the inactive kinase domain adopts a conformation similar to that of cyclin-dependent kinases and Src kinases. The autoinhibitory mechanism of ZAP-70 is, however, distinct and involves interactions between the regulatory segment and the hinge region of the kinase domain that reduce its flexibility. Two tyrosine residues in the SH2-kinase linker that activate ZAP-70 when phosphorylated are involved in aromatic-aromatic interactions that connect the linker to the kinase domain. These interactions are inconsistent with ITAM binding, suggesting that destabilization of this autoinhibited ZAP-70 conformation is the first step in kinase activation.

INTRODUCTION

The protein tyrosine kinase ZAP-70 (zeta-chain-associated protein of 70 kDa; Chan et al., 1992) is crucial for signaling by the T cell antigen receptor (TCR; Arpaia et al., 1994; Chan et al., 1994; Elder et al., 1994; Negishi et al., 1995). The TCR is activated by antigenic peptides that are displayed by the major histocompatibility complex (MHC) molecule on the surface of antigen-presenting cells. TCR engagement results in the tyrosine phosphorylation of multiple proteins, which is followed by activation of diverse signaling pathways and, eventually, by alterations in gene expression, T cell proliferation, and the secretion of cytokines.

The TCR has no intrinsic catalytic activity. Instead, activated TCRs recruit ZAP-70, which phosphorylates proteins such as LAT (linker for the activation of T cells) and SLP-76 (Src homology 2-domain-containing leukocyte protein of 76 kDa). These proteins then serve as scaffolds to organize signaling complexes essential for onward signal transmission (Horejsi et al., 2004; Koretzky et al., 2006).

The key step that links TCR activation to ZAP-70 is the phosphorylation by Src family kinases (e.g., Lck) of sets of paired tyrosine residues (called ITAMs, for immunoreceptor tyrosine-based activation motifs) in the cytoplasmic tails of the TCR ζ and CD3 chains. ITAMs share the sequence motif YXX(L/I)$X_{(6-8)}$YXX(L/I), in which the two tyrosine residues are the sites of phosphorylation, and "X" denotes variable residues. ZAP-70 contains two tandemly arranged SH2 domains (see Figure 1A for a schematic diagram). The two SH2 domains are coupled tightly and recognize phosphorylated ITAM motifs with high specificity and affinity (Hatada et al., 1995). Signaling by the B cell receptor and other ITAM-containing receptors (i.e., Fc receptors and NK receptors) involves very similar early events (van Oers and Weiss, 1995). For instance, when the B cell receptor is stimulated, the tyrosine kinase Syk, a close relative of ZAP-70, is recruited to the receptor after phosphorylation of its ITAM sequences by Src family kinases.

Little is known at present about the mechanism by which recruitment of ZAP-70 to ITAMs triggers its activation. The structure of full-length ZAP-70 has not yet been reported. The phosphorylation of two pairs of tyrosine residues in ZAP-70 is crucial for the activation process (Brdicka et al., 2005; Chan et al., 1995; Di Bartolo et al., 1999; Wange et al., 1995; Watts et al., 1994; Wu et al., 1997). One pair (Tyr492 and Tyr493 in human ZAP-70) is located in the activation loop of the kinase domain, and the phosphorylation of these tyrosines is most likely initiated by Lck (Chan et al., 1995; Watts et al., 1994) or by transautophosphorylation (Brdicka, et al., 2005). The structure of the isolated kinase domain of ZAP-70 suggests that, as for other kinases, phosphorylation of the

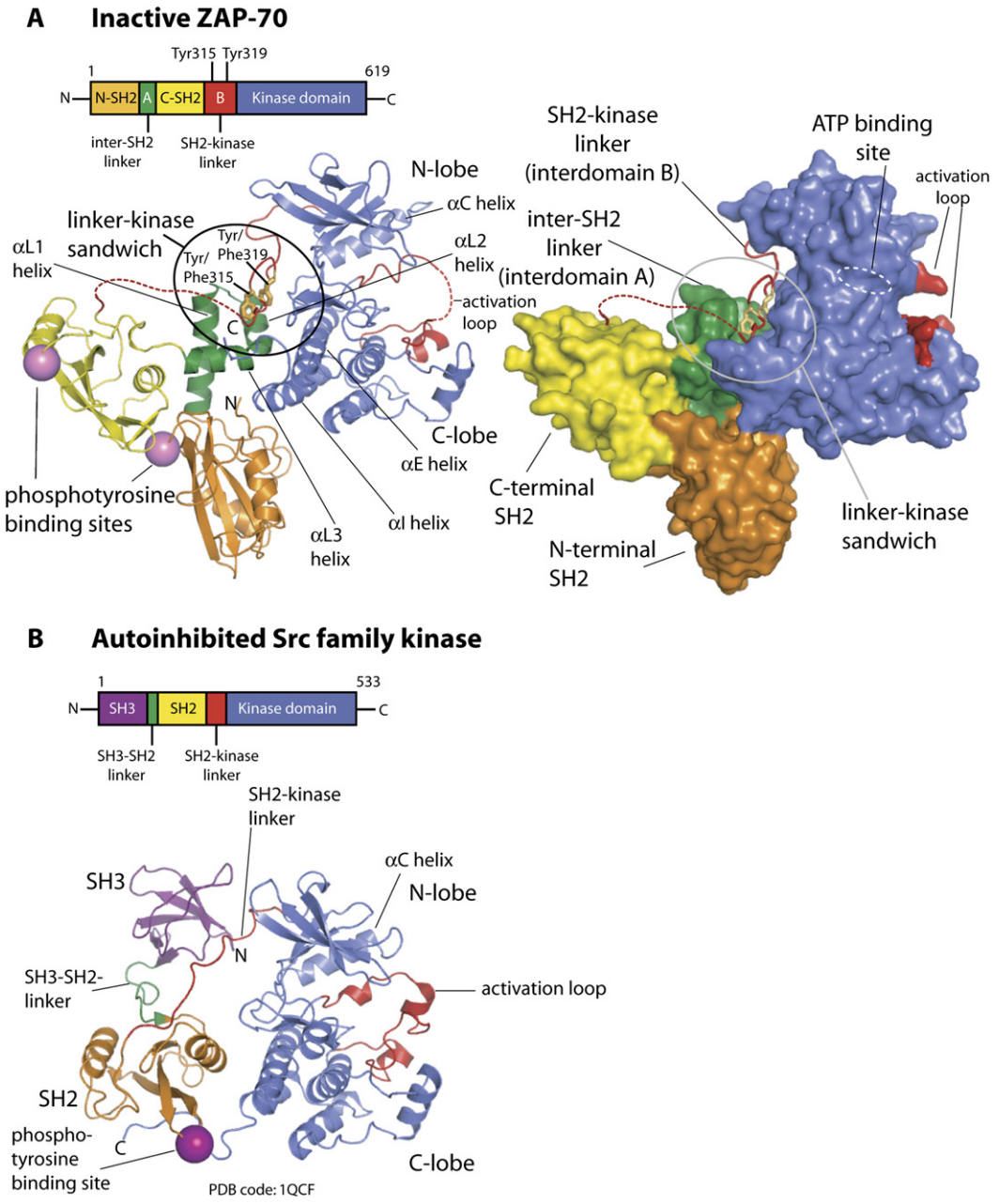

Figure 1. Structural Organization of Inactive ZAP-70 and Comparison with Autoinhibited Src Family Kinases

(A) Domain organization (top) and crystal structure (bottom) of inactive ZAP-70. The kinase domain, SH2-kinase linker, inter-SH2 linker, C-terminal SH2 domain, and N-terminal SH2 domain are shown in blue, red, green, yellow, and orange, respectively. The two phosphotyrosine-binding sites are indicated with magenta spheres. Disordered regions are depicted as dotted lines.

(B) Domain organization (top) and structure (bottom) of autoinhibited Src family kinases. The structure shown here is that of inactive Hck (PDB code 1QCF; Schindler et al., 1999).

activation loop would stabilize the active conformation (Jin et al., 2004).

The other two critical tyrosine residues (Tyr315 and Tyr319) are located in the SH2-kinase linker. These are phosphorylated upon recruitment of ZAP-70 to the TCR, most likely by Lck (Brdicka et al., 2005; Williams et al., 1999) or by ZAP-70 itself (Di Bartolo et al., 1999). While Tyr315 or Tyr319 may serve as SH2 docking sites when phosphorylated, deletion of most of the SH2-kinase linker (including Tyr315 and Tyr319) preserves at least some ZAP-70 function (Zhao et al., 1999). Thus, the SH2-kinase linker is likely to be part of an autoinhibitory mechanism.