SH3 domain ligand binding: What's the consensus and where's the specificity?

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An increasing number of SH3 domain–ligand interactions continue to be described that involve the conserved peptide-binding surface of SH3, but structurally deviate substantially from canonical docking of consensus motif-containing SH3 ligands. Indeed, it appears that that the relative frequency and importance of these types of interactions may have been underestimated. Instead of atypical, we propose referring to such peptides as type I or II (depending on the binding orientation) non-consensus ligands. Here we discuss the structural basis of non-consensus SH3 ligand binding and the dominant role of the SH3 domain specificity zone in selective target recognition, and review some of the best-characterized examples of such interactions.

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1. Introduction

SH3 domain is a small protein interaction module composed of a β-sandwich consisting of five strands connected by three loops and a short 310 helix (Fig. 1). The pioneering early studies defined the ligand sequence PxXP as the minimal consensus target site for SH3 domain binding, and revealed how the PxXP motif is accommodated by two different Xp dipeptide-binding pockets on the SH3 surface [1,2]. Moreover, it was found that such PxXP motif-containing peptides could be docked in two opposite orientations defined by the relative positioning of a positively charged residue (+XpXpXp or XpXpXp+) interacting with a negatively charged third cleft on the SH3 peptide-binding surface [3,4]. This third cleft was named the specificity pocket. Recognition of these Class I and Class II consensus SH3 binding motifs has since helped the identification of the interaction partners for many SH3 domains, and dominated the thinking in this field.

However, an increasing number of divergent SH3 domain target peptides, often referred to as atypical binding motifs, have been identified. Thus, it would probably be more appropriate to call such peptides non-consensus ligands, and to restrict the term atypical for the less common and fundamentally different interactions that involve entirely different surfaces on the SH3 domain (for examples, see [5,6]). Of note, excluding interactions involving additional tertiary contacts (e.g. Hck-SH3/Nef binding [7]), SH3/ligand complexes that show unusually strong affinity or distinct selectivity are usually, if not always, based on a non-consensus binding motif.

Unlike the canonical PxXP motif-based ligands, peptides containing non-consensus SH3 binding motifs do not always adopt a polyproline type-II (PPII) helical conformation, and may not occupy both xP-binding pockets of the SH3 domain (see Figs. 2 and 3). On the other hand, a characteristic feature of the non-consensus motifs is their extensive use of contacts with the SH3 surface that typically contains the negatively charged specificity pocket (see Figs. 2 and 4). Compared to the orientation-defining salt bridges provided by the specificity pocket upon Class I and II consensus binding, elaborate sets of contacts with side chain atoms from several residues in non-consensus ligands can provide significant additional affinity and specificity to these interactions. Indeed, the role of such contacts can dominate over those involving the xP-binding pocket interface of the SH3 domain.

The SH3 surface that contributes to these affinity/specificity-determining interactions is formed by a shallow valley above the β3 and β4 strands, flanked by the far end of strand β2/n-Src loop and the tip of RT loop (see Fig. 1). In many SH3 domains this surface consists of more than one distinct subpocket, which together with, or in many cases instead of, a canonical acidic pocket accommodate ligand residues located N- or C-terminally (in type I or II binding, respectively) of the xP-pocket-contacting region of the peptide (see peptide illustrations in Fig. 2). Because of its structural complexity and coverage of a large SH3 surface area, we prefer to use the term specificity zone to make a clear distinction to the traditional concept of a specificity pocket.
The divergent strategies for combinatorial use of the xP pocket region and the specificity zone exhibited by different consensus or non-consensus ligands are illustrated in Fig. 2. Remarkably, common sets of molecular contacts with the SH3 specificity zone have evolved as modules that can appear in peptides containing a canonical PPII-helical PxxP motif as well as in peptides that interact with the SH3 xP-pocket region via other strategies. A striking example of this is provided by the RxxK motif of the HPK1 and SLP-76 peptides that docks to the specificity zone of GADS SH3 domain in a similar manner, despite completely different modes of GADS xP-pocket surface recognition by these peptides [12–14]. On the other hand, even in the case of a single ligand peptide, the modes of xP pocket and specificity zone recognition can differ between two SH3 domains. This is clearly exemplified by binding of the aminoterminal SH3 domain of Nck and the Eps8L1 SH3 domain to CD3ε [15,16,21]. In both complexes much of the specificity and affinity of binding is provided by an interaction of a DY motif in the ligand with the SH3 specificity zone, whereas only Nck SH3 binding involves canonical accommodation of the CD3ε peptide as a PPII ligand on the xP pocket surface of the SH3 domain.

The binding determinants in the ligands that interact with the SH3 specificity zone can be quite complex and adopt distinct secondary structures. Presentation of such specificity determinants in the context of a 3₁₀ helix can be observed in many cases, including the indicated high-affinity interactions of GADS [12–14], Csk [10], and pPIX SH3 [18–20] domains with their non-consensus ligands, whereas the remarkable binding strength (24 nM) of the carboxyterminal SH3 domain of p67phox with a proline-rich region of p47phox (another subunit of the NADPH oxidase) depends on an interaction between a helix-turn-helix structure in the p47phox ligand with the specificity zone of the p67phox SH3 domain [11]. Some, but not all of these interactions are complemented by canonical ionic interactions between a basic residue in the ligand an acidic pocket in the specificity zone of the SH3 domain (see Fig. 2).

Interestingly, the PPII helical conformation is not restricted to the xP-pocket region contacts by the ligands, but is also utilized for contacts with the specificity zone [20,21]. An extreme case of this is the complex between the IRTKS SH3 domain and its ligand EspFU. This bacterial peptide contains a typical PPII-helical PxxP motif to interact with the xP pocket region of IRTKS SH3 as a Class I ligand, but exploits a unique strategy for interacting with the specificity zone. The specificity zone of IRTKS SH3 contains two hydrophobic clefts, which resemble the xP pockets and accommodate the aminoterminal part of the EspFU peptide in a manner that phenocopies a bona fide PPII-helical PxxP consensus peptide interaction [21].
Although the specificity zone is critical in providing distinct selectivity for SH3 interactions the xP pocket surface can also contribute specificity for binding. For example, the positioning of the highly conserved W37 residue is slightly different in SH3 domains complexed with Class I vs. II consensus ligands. Depending on the type of residue at position 55 this movement of W37 may be hindered, thereby allowing binding only to Class II peptides or to a subset of Class I ligands presenting Leu-Pro dipeptides to the SH3 xP pockets [24].

Examples of more distinguishing contacts with the xP pocket surface can be observed with SH3 ligands that do not contain PPII helical conformation, such as the complex involving the βPix SH3 domain and its target peptide in p21-activated kinase-1 [18] and 2 [19]. Although SH3 domains like GADS (see Fig. 3) or Fyn [25] may bind both canonical PxxP ligands as well as non-PPII helical peptides, the anatomy of the xP pockets can favor one of these. As explained in more detail in the legend for Fig. 3, this is the case with the SH3 domains of Eps8L1 and GADS, which prefer non-PPII ligands because of their unusual amino acid residues in certain conserved xP-pocket-forming positions.

Other informative examples of n-Src loop residue modifications that contribute to ligand-specific contacts at the specificity zone are provided by the Csk SH3 in complex with PEP-3BP1 [10] and the C-terminal SH3 domain of p67phox in complex with p47phox [11]. In both cases, the ligand peptides bind in the minus-orientation, and canonical contacts with the SH3 xP pockets and an acidic pocket in the specificity zone. However, for additional contacts with the specificity zone, both peptides form a helical structure, although the exact roles of these secondary structures are quite different. The PEP-3BP1 establishes a 310 helix presenting a hydrophobic isoleucine residue that clamps around a finger in the n-Src loop formed by K33 of Csk SH3. This interaction is complemented by a valine in PEP-3BP1 that inserts into a hydrophobic cavity (circled in yellow) in the specificity zone of Csk SH3 formed by A30 and T32. In the case of p67phox–p47phox complex, the

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**SH3**

<table>
<thead>
<tr>
<th>SH3</th>
<th>Ligand</th>
<th>Orientation</th>
<th>Ligand architecture</th>
<th>PDB entry</th>
<th>Ref.</th>
</tr>
</thead>
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<td>+/-</td>
<td>N/C PPPI C/C</td>
<td>1RLQ 1SEM</td>
<td>[3, 4]</td>
</tr>
<tr>
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<td>C3G PPPALP4KR</td>
<td>-</td>
<td>N PPPI C</td>
<td>1CKA</td>
<td>[8]</td>
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<td>+</td>
<td>C PPPI N</td>
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<td>-</td>
<td>N PPPI C</td>
<td>1JEG</td>
<td>[10]</td>
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<tr>
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<td>p47phox PEPVPPPPSADILINRCSESTKRRKL</td>
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<td>N PPPI C</td>
<td>1K4U</td>
<td>[11]</td>
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<td>-</td>
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<tr>
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<td>1OE8 1H3M</td>
<td>[13, 14]</td>
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<tr>
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<td>C PPPI C</td>
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<td>+</td>
<td>C PPPI N</td>
<td>2KKC</td>
<td>[21]</td>
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**Fig. 2.** Diverse strategies for SH3 ligand binding. Peptide conformations observed in a selected set of informative SH3 / ligand structures [3,4,8–21] are schematically shown together with key data on these interactions. The letters N and C are shown in parentheses after the name of proteins containing two SH3 domains to indicate whether the amino- or carboxyterminal SH3 is meant. The symbols Φ and Ω refer to hydrophobic and aromatic amino acid residues, respectively, whereas “v” is a positively charged residue (Arg or Lys), and x is any amino acid. Amino acid sequences of the relevant regions of the ligands are shown under the origin of these peptides. The relative orientation of the peptides in the SH3/ligand complexes is indicated by a plus (+) or a minus (−), and also by N and C marking the amino- and carboxytermini of the schematically illustrated ligand peptides. Since the majority of ligands included in the figure bind to their cognate SH3 domains as Class II ligands, we have chosen to depict the N-termini of such minus-orientation peptides as pointing to the left and their C-termini pointing to the right. When a peptide is interacting with the SH3 xP pocket surface as a typical PPII-helical, the corresponding xPxxP sequence is underlined in the sequence, and depicted in the peptide illustrations as a rounded white box marked PPi. Of note, the EspF peptide is using an identical PPi structure also for its interaction with the specificity zone of the IRTKS SH3 domain. However, in this case the first xP pocket in the specificity zone is dedicated for binding an IP-dipeptide, thus providing specificity for this high affinity (500 nM) interaction. On the other hand, some ligands do not form any PPi helix, and instead interact with the SH3 xP-pocket via very different strategies. When the complex involves an interaction of a canonical positively charged residue of the ligand with an acidic pocket in the specificity zone of the SH3 domain, this residue has been colored red in the sequence, and is indicated as a yellow/orange triangle in the peptide illustration. The orange triangle denotes the special case of the aminoterminal SH3 of the Crk/CrkL proteins. As revealed by the Crk/C3G complex structure these SH3 domains specifically select Class II ligands with a lysine as the positively charged consensus residue, which they coordinate in an unusual and tight manner by a set of three acidic SH3 residues [8]. The other residues in the ligand peptides that make key contacts with the SH3 specificity zone are colored green, and the structural elements presenting these residues are marked as various colored symbols in the peptide drawings.
32-residue of the p47phox peptide establishes a helix-turn-helix structure in the specificity zone p67phox SH3. The critical SH3 residues are I50 in b4 strand and V32 in the n-Src loop that make direct contacts with the α-helices in p47phox peptide.

2. Conclusions and perspectives

Here we define non-consensus SH3 ligands as peptides that do not contain a PPII-helical PxxP motif and/or depend on specificity determinants more complex than the canonical basic residue of Class I and II consensus peptides. Instead of an acidic pocket that accommodates such a basic residue, the specificity determinants of non-consensus ligands typically make more extensive contacts with an overlapping but more complex surface in their cognate SH3 domains, which we refer as the specificity zone. Consequently, the binding affinity and selectivity of interactions involving non-consensus SH3 ligands can be substantially greater than observed for consensus peptides. On the other hand, from the distinct and variable nature of such specificity zone contacts, it also follows that non-consensus SH3 ligands cannot be readily predicted from protein sequence data. It is possible that the relative prevalence of non-consensus ligands is significantly higher than currently appreciated, and the perceived dominance of Class I or Class II motifs rather reflects the historical focus on Src-like proteins in studies leading to the identification of the SH3 domain. Indeed, the majority of the approximately 300 SH3 domains encoded by the human genome are still lacking a characterized ligand. The ability to create random peptide libraries of increasing complexity and peptide length, together with an improved capacity to characterize ligand preferences for a large number of different SH3 domains...
domains using modern high-throughput technologies may soon shed more light into this question (see \[26–28\]). Finally, while mediating tight and specific contacts with SH3 ligands, the complex and variable surface of the specificity zone also has much more potential for drug targeting than the flat, hydrophobic, and structurally conserved xP pockets. Therefore, the specificity zone should be considered a prime target in the efforts to develop pharmacological inhibitors against disease processes mediated by SH3 domain–guided protein interactions.

Fig. 4. Anatomy of the specificity zone – ligand interface in selected SH3 domain – peptide complexes. Shown are six different SH3 domains in complex with Class I and II non-consensus peptides to illustrate contacts in the specificity zone that enhance affinity and selectivity of SH3 ligand binding. Insulin receptor tyrosine kinase substrate (IRTKS) SH3 in complex with the pathogen-encoded peptide EspFU\[21\], Abelson kinase (Abl) SH3 in complex with p41 [9], and βPIX SH3 in complex with AIP4 peptide [20] all represent Class I interactions (ligand binding in plus-orientation), but their binding preferences are very different. In IRTKS and Abl, the canonical acidic residue at position 17 is replaced by leucine and threonine, respectively, compromising their binding to classical RxxPxxP ligands, whereas βPIX contains a glutamate at position 17, and accordingly accommodates a typical basic ligand residue in this slot. However, the specificity zones in Abl, βPIX and IRTKS contain additional slots for enhanced ligand binding specificity and affinity. In all these three SH3 domains the first additional binding pocket (circled in yellow) is hydrophobic and is established by the highly conserved W37 residue together with W50 from j4 strand. This pocket interacts with a proline residue in the ligand. The second additional pocket found in IRTKS and βPIX (circled in green) helps to further increase their ligand binding specificity. Hydrophobic residues rarely encountered at the tip of j2 strand and in j3 strand (positions 30 and 39) render this second pocket in IRTKS highly hydrophobic, and strongly favors docking of an IP dipeptide. Thus, through the combined action of the two hydrophobic pockets in the specificity zone and the two canonical xP pockets IRTKS specifically recognizes peptide ligands with the consensus IPXxxXDIxXP [21]. By contrast in βPIX the position 39 contributing to the second specificity zone pocket is negatively charged by glutamate, and accommodates a positively charged arginine of the AIP4 peptide [20]. Nevertheless, as already discussed in the case of xP pocket usage in Fig. 3, βPIX SH3 binds also non-consensus Class II ligands, and forms ββ helix upon interaction with the specificity zone, as highlighted for βPIX SH3 – PAK2 complex [19].

References


