The exquisite specificity exhibited by many proteins for their respective ligands can often be attributed to particular structural features within the binding site. The Src homology 2 (SH2) domain of interleukin-2 tyrosine kinase (Itk) contains overlapping binding sites capable of mediating binding to two distinct ligands: a phosphotyrosine-containing peptide and the Itk Src homology 3 (SH3) domain. We have previously demonstrated that the Asn 286-Pro 287 imide bond in the Itk SH2 domain adopts both the cis and trans conformations in solution. Exchange between the conformers is slow on the NMR time scale, leading to the appearance of doubled resonances in NMR spectra for 35 of the 109 SH2 residues (Figure 1b). The structural changes induced by isomerization of the peptidyl prolyl imide bond in the Itk SH2 domain modulate its affinity for both of its ligands. We now report a quantitative study of the equilibria governing the peptidyl prolyl cis/trans isomerization and concomitant ligand binding to the Itk SH2 domain. Hereafter, we will refer to the cis and trans imide bond containing SH2 conformers as the cis and trans conformers, respectively.

The conformer-specific nature of Itk SH2 ligand recognition is evident in NMR spectra of the protein/ligand complexes. Addition of phosphopeptide to the Itk SH2 domain shifts the equilibrium to favor the trans conformer, resulting in changes in the volumes of the NMR peaks corresponding to each conformer (Figure 1c). Also, ligand-induced chemical shift changes are larger for cross-peaks corresponding to residues in the trans conformer than for those corresponding to residues in the cis conformer. In contrast, binding of the Itk SH3 domain to the SH2 domain shifts the cis/trans ratio to favor the cis conformer (Figure 1d). In this case, shifts in the positions of cross-peaks corresponding to the cis conformer (but not those of the trans conformer) are observed in the HSQC spectrum of the Itk SH2 domain to which recombinant Itk SH3 domain has been added. To our knowledge, this is the first demonstration of ligand recognition that is governed by the conformation of a single prolyl imide bond within a folded protein. However, a qualitative analysis of the data cannot establish that the trans SH2 conformer has no affinity for the Itk SH3 domain or that the cis conformer cannot bind phosphopeptide. We have therefore developed a method of analyzing chemical shift perturbation and cross-peak volumes to measure the binding affinities of both ligands for each SH2 conformer.

The simplest model for SH2 binding to either ligand is given by eq 1:

$$P + L \rightleftharpoons K_c PL$$

where P is the protein (Itk SH2), L the ligand (phosphopeptide or

![Figure 1](image-url)
the observed association constants (Table 1), we can compute \( f_b \) for each point of the titration, while \( f_c \) is measured by integrating the volumes of the cis and trans cross-peaks of Itk SH2 residues affected by isomerization. A plot of \( f_c \) versus \( f_b \) yields a straight line (Figure 3). If the Itk SH3 domain bound exclusively to the cis Itk SH2 conformer, then \( f_b = 1 \) would result in \( f_c = 1 \). Similarly, for the phosphotyrosine titration, at \( f_b = 1 \) a value of \( f_c = 0 \) is expected if only the trans SH2 conformer is able to bind phosphopeptide. This is not the case for either the phosphopeptide or Itk SH3 ligations (Figure 3, parts a and b, respectively), indicating that both SH2 conformers (cis and trans) have measurable affinity for each of the ligands as represented in the following equilibrium model (eqs 2–4):

\[
P^t + L \rightleftharpoons PL^t \quad (2)
\]

\[
P^c + L \rightleftharpoons PL^c \quad (3)
\]

\[
P^t \rightleftharpoons P^c \quad (4)
\]

where the \( t \) and \( c \) superscripts refer to the trans and cis conformers, respectively. The trans to cis interconversion constant \( K' \) is given by the integrated volume ratio of the cis and trans cross-peaks of free Itk SH2 (0.64 ± 0.06, see Figure 1). The equilibrium model given by eqs 2–4 predicts a relationship between \( f_c \) and \( f_b \), which can be used to determine the equilibrium constants \( K_c \) and \( K_t \) (derivation in the Supporting Information):

\[
f_c = \frac{K'(K_c - K_t)}{(1 + K')(K_c + K_t K')} f_b + \frac{K'}{1 + K'} \quad (5)
\]

\( K_c \) and \( K_t \) are then obtained by fitting eq 5 to the cis/trans peak integral data of five cis/trans pairs (Figure 3). The solid line in Figure 3 represents the fitted model, and the affinities of the cis and trans SH2 conformers for both ligands are reported in Table 1.

**Table 1.** Observed, Cis, and Trans Association Constants for the SH2 Ligands

<table>
<thead>
<tr>
<th>Ligand</th>
<th>( K_c ) (mM(^{-1}))</th>
<th>( K_t ) (mM(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphopeptide</td>
<td>2.9 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>SH3</td>
<td>1.0 ± 0.2</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>SH2</td>
<td>4.0 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
</tbody>
</table>

Quantitative analysis of the NMR data reveals the extent to which ligand-binding affinities are modulated by isomerization between Itk SH2 conformers. The cis SH2 conformer exhibits a 3.5-fold higher affinity for the Itk SH3 domain compared to binding of the trans SH2 conformer to the same ligand. Likewise, the trans SH2 conformer binds phosphopeptide with a 4-fold greater affinity than the corresponding cis SH2 conformer. Thus, for the Itk SH2 domain, cis/trans isomerization of a single prolyl imide bond affords this small domain the ability to control the relative binding affinities for distinct ligands during cell signaling. Additional examples of protein-binding modules that exhibit recognition of distinct targets have been previously reported.\(^5\)\(^6\) The Itk SH2 domain represents the first example of dual ligand recognition prolined cis/trans isomerization within the receptor. Given the intrinsic nature of proline isomerization and the modest energy barrier between conformers, proline cis/trans isomerization may be a general mechanism allowing for diversity in protein recognition.

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**Supporting Information Available:** Derivations of the governing equations for the equilibrium model (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

**References**


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