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Ligand Specificity Modulated by Prolyl Imide Bond Cis/Trans Isomerization in the Itk SH2 Domain: A Quantitative NMR Study

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Abstract

The Src homology 2 (SH2) domain of interleukin-2 tyrosine kinase (Itk) binds two separate ligands: a phosphotyrosine-containing peptide and the Itk Src homology 3 (SH3) domain. Binding specificity for these ligands is regulated via cis/trans isomerization of the Asn 286–Pro 287 imide bond in the Itk SH2 domain. In this study, we develop a novel method of analyzing chemical shift perturbation and cross-peak volumes to measure the affinities of both ligands for each SH2 conformer. We find that the cis imide bond containing SH2 conformer exhibits a 3.5-fold higher affinity for the Itk SH3 domain compared with binding of the trans conformer to the same ligand, while the trans conformer binds phosphopeptide with a 4-fold greater affinity than the cis-containing SH2 conformer. In addition to furthering the understanding of this system, the method presented here will be of general application in quantitatively determining the specificities of conformationally heterogeneous systems that use a molecular switch to regulate binding between multiple distinct ligands.

Disciplines

Biochemistry, Biophysics, and Structural Biology | Molecular Biology

Comments

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Ligand Specificity Modulated by Prolyl Imide Bond Cis/Trans Isomerization in the Itk SH2 Domain: A Quantitative NMR Study

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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[1] (Figure 1a). 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.[2,3] 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[2]. 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:

$$P + \mathrm{L} \overset{K_o}{\rightleftharpoons} \mathrm{PL} \qquad (1)$$

where P is the protein (Itk SH2), L the ligand (phosphopeptide or Itk SH3), PL the protein/ligand complex, and K_o the observed association constant. Both Itk SH2/ligand binding events are in the fast exchange regime such that the resonance frequencies of reporter residues (residues involved in binding of the ligand) are a population-weighted average of the bound and unbound frequencies. The dependence of resonance frequency on ligand concentration can be used to extract K_o. It is incorrect, though, to use an exchange-doubled resonance as a reporter, because the concentrations of the cis and trans conformers will vary over the course of the titration. Therefore, five well-resolved SH2 cross-peaks unaffected by proline isomerization (N241, Y292, V311, F312, and L318) were used to determine K_o for Itk SH2 phosphopeptide binding (Figure 2a, Table 1). In the case of Itk SH3 binding, all Itk SH2 peaks that exhibit measurable chemical shifts are affected by the cis/trans isomerization of Pro 287. K_o was therefore measured for this system by analysis of the NMR data acquired using the reverse labeling scheme (unlabeled SH2 domain titrated into ^{15}N-labeled SH3 domain). The concentration dependence of the normalized chemical shifts for five SH3 cross-peaks (Y180, W208, W209, A221, and S223) are shown in Figure 2b, and K_o for the association is reported in Table 1.

To determine the affinities of the SH2 conformers for each of the two ligands, we analyzed the fraction of Itk SH2 domain bound to ligand (f_b, concentration of ligand-bound SH2 divided by total SH2 concentration) and the fraction in the cis conformer (f_c, concentration of cis imide bond-containing SH2—in both bound and unbound forms—divided by total SH2 concentration). Using

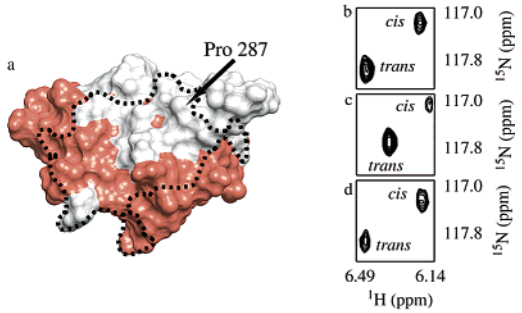

Figure 1. (a) Surface representation of the cis SH2 domain. The residues whose chemical shifts change upon Itk SH3 binding are white, while those unaffected are orange. The region bound by the dotted line indicates the residues that are affected by binding to the phosphotyrosine-containing peptide ADpYEPPPSNDE. The position of Pro 287 is indicated. (b) Select region of the heteronuclear single quantum coherence (HSQC) spectrum showing the doubled resonances for Lys 258 of the Itk SH2 domain. The cross-peak corresponding to the trans conformer is downfield compared to the cis in both the ^{1}H and ^{15}N dimensions. The integrated volume ratio of the cis and trans cross-peaks (I_c/I_t) is equal to 0.64 ± 0.06. (c) Corresponding region for ^{15}N-labeled Itk SH2 domain following addition of a 20-fold excess of phosphopeptide ($I_c/I_t = 0.22 \pm 0.07$). (d) Corresponding region for ^{15}N-labeled Itk SH2 domain following addition of a 7-fold excess of the Itk SH3 domain ($I_c/I_t = 1.60 \pm 0.19$).

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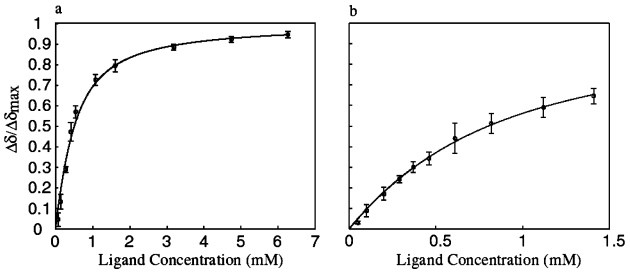

Figure 2. Concentration dependence of normalized chemical shifts for (a) titration of phosphopeptide into ^{15}N-labeled Itk SH2 domain (0.32 mM) and (b) titration of unlabeled Itk SH2 domain into ^{15}N-labeled Itk SH3 (0.32 mM). In both cases, the curves depict the best fit of the model defined by eq 1 to the data (see Supporting Information). Error bars represent two standard deviations about the mean.

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

	Itk SH2 binding phosphopeptide	Itk SH2 binding SH3
K_o (mM^{-1})	2.9 ± 0.1	1.5 ± 0.1
K_c (mM^{-1})	1.0 ± 0.2	2.6 ± 0.3
K_t (mM^{-1})	4.0 ± 0.2	0.8 ± 0.2

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 titrations (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):

$$P^t + L \overset{K_t}{\rightleftharpoons} P^tL \tag{2}$$

$$P^c + L \overset{K_c}{\rightleftharpoons} P^cL \tag{3}$$

$$P^t \overset{K'}{\rightleftharpoons} P^c \tag{4}$$

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):

$$f_c = \left(\frac{K'(K_c - K_t)}{(1 + K')(K_t + K_cK')}\right) f_b + \frac{K'}{1 + K'} \tag{5}$$

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.

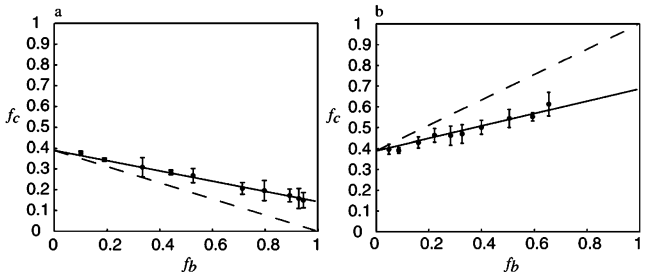

Figure 3. Plot of the fraction of cis Itk SH2 (f_c) versus the total fraction bound of ligand (f_b) for (a) titration of phosphopeptide into a sample containing ^{15}N Itk SH2 domain and (b) titration of the Itk SH3 domain into a sample containing ^{15}N Itk SH2 domain. The well-resolved resonances corresponding to residues T256, K258, G257, F278, and G260 were used in (a) and C288, K258, G257, K290, and G260 were used in (b). Error bars represent two standard deviations about the mean. The solid line is the fit of eq 5 to the data. The dotted line represents the expected fraction of cis if phosphopeptide exclusively bound the trans conformer ($K_c = 0$) in (a) or Itk SH3 exclusively bound the cis conformer ($K_t = 0$) in (b).

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 controlled by proline 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.

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