Ligand-specific conformational change drives interdomain allostery in Pin1

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Abstract

Pin1 is a two-domain cell regulator that isomerizes peptidyl-prolines. The catalytic domain (PPlase) and the other ligand-binding domain (WW) sample extended and compact conformations. Ligand binding changes the equilibrium of the interdomain conformations, but the conformational changes that lead to the altered domain sampling were unknown. Prior evidence has supported an interdomain allosteric mechanism. We recently introduced a magnetic resonance-based protocol that allowed us to determine the coupling of intra- and interdomain structural sampling in apo Pin1. Here, we describe ligand-specific conformational changes that occur upon binding of pCDC25c and FFpSPR. pCDC25c binding doubles the population of the extended states compared to the virtually identical populations of the apo and FFpSPR-bound forms. pCDC25c binding to the WW domain triggers conformational changes to propagate via the interdomain interface to the catalytic site, while FFpSPR binding displaces a helix in the PPlase that leads to repositioning of the PPlase catalytic loop.

Introduction

Many proteins are organized into multiple domains with the potential for interdomain crosstalk. The function and activity of one domain can be modulated through the structure and dynamics of another domain, what we thus term “interdomain allostery”. In addition, the structure and dynamics of the entire system may change upon environmental change (i.e. substrate binding). While interdomain allostery can be demonstrated by comparing the activity of the individual domains to the whole multi-domain system, it is challenging to directly probe the cascades of conformational changes leading to allostery.

Extensive work has focused on investigating interdomain allostery through the two-domain mitotic regulator Pin1^{1–7}. Pin1 regulates other proteins through isomerization of the peptide bonds of prolines that leads to the recycling of phosphoproteins, regulating other post-translational modification, and enhancing or preventing protein degradation⁸. Pin1 consists of the WW binding domain (residues 1-39) connected to the peptidyl-prolyl isomerase (PPlase) domain (residues 50-163) via a flexible linker (Figure 1 and **Supplemental Figure 1b**). Pin1’s PPlase domain isomerizes the peptide bonds of prolines that are immediately preceded by either a phosphorylated serine or threonine (pS/T-P motif). While the PPlase can catalyze both *cis-trans* or *trans-cis* reactions, the WW domain binds *trans*-specifically. The domains have been shown to populate both a “compact” state with a specific interdomain (ID) interface as well as a dispersed “extended” state where the two domains can tumble semi-independently as displayed in Figure 1. Our recent work suggests that apo Pin1 occupies a compact and extended state in a 70:30 ratio⁹.

The primary ligands that have been biophysically evaluated are FFpSPR (peptide sequence) and pCDC25c (with peptide sequence EQPLpTPVTDL). FFpSPR is a more soluble variant of another artificial ligand (Pintide with peptide sequence WFYpSPR) that has high PPlase efficiency with minimal sequence¹⁰. The pCDC25c ligand is a peptide derivative from the pT48-P49 site of the mitotic phosphatase CDC25c¹¹. An early study of interdomain mobility compared tumbling times (τ_c) of apo Pin1 and Pin1 complexed to various ligands. The study concluded that Pintide restricts the flexibility of

the two domains (i.e. induces a more compact state), while pCDC25c increases the flexibility⁷. Further NMR studies involving chemical shift perturbations (CSPs) and order parameters quantifying the motional restriction of covalent bonds have supported the reduced interdomain contact caused by pCDC25c^{1,2,5,12}, but the proposed compaction due to FFpSPR binding is not supported by published data⁶ (Figure 1). As the divergent CSPs mainly occur on the interdomain interface (**Supplemental Figure 1**), they indeed indicate that these ligands cause distinct allosteric effects and interdomain orientations.

It has been established that the WW domain can allosterically regulate the activity of the PPlase^{1,2}. While only the PPlase domain is catalytically active, mutagenesis studies have shown that the WW domain is essential *in vivo*¹³. Yet, the isolated PPlase domain displays higher catalytic activity than the full-length protein for both FFpSPR and pCDC25c ligands². Therefore, WW domain contact alters the properties of the PPlase catalytic site, which is located on the opposite side of the PPlase domain than the interface with the WW domain. Overall, these observations support interdomain allostery coupled to intradomain allostery. Interdomain communication and dynamic allostery are also supported by the fact that ligand-binding reduces methyl group flexibility along a conduit linking the ID interface to the catalytic site⁵. The exact conduit residues and degree of change shows some variance depending on the ligand and its effect on the interdomain contact^{2,6}. More evidence of interdomain allostery comes from the expansive studies on the I28A mutation in the WW domain interface. This mutation in the WW domain increases the activity of the PPlase with evidence supporting the stabilization of the extended state^{1,12,14}. A conformational selection-driven allosteric model has been proposed that explains why PPlase activity is negatively regulated by interdomain contact^{1,2}. Both extended and compact states exist in apo Pin1; shifting the equilibrium to favor the extended state by either mutating the interface, binding to pCDC25c, or through deleting the WW domain causes an increase in catalysis¹. In addition, a recent study shows that pCDC25c binding causes compaction within the WW domain while the interdomain distance increases¹⁵. In contrast, other ligands, including FFpSPR, do not have the same effect on the interdomain contact^{4,16}. An extensive molecular dynamics study suggests that two pathways mediate the interdomain allosteric regulation of ligand FFpSPR: “path 1” occurs through the ID interface that preexists in apo Pin1, and “path 2” connects the two domains, specifically the WW domain and the long α 1 helix of the PPlase domain, upon ligand binding⁴. All this data establishes that ligand binding changes the intradomain structure and interdomain orientation, and that the specific ligand sequence has distinct effects on Pin1.

Previous work has extensively described signatures of allostery in Pin1, yet to date the structure of ligand-bound, full-length Pin1 in solution has not been determined. Though ligand-bound crystal structures exist¹⁷, they show Pin1 only occupying a compact configuration. We recently published a protocol for elucidating the coupling of inter- and intradomain spatial sampling of multi-domain proteins using magnetic resonance⁹: first, we calculated multi-state structural ensembles of apo Pin1 from exact Nuclear Overhauser Enhancements (eNOE) and scalar couplings restraining the intradomain structure, whereas paramagnetic relaxation enhancement (PRE), interdomain NOEs and residue dipolar couplings (RDC) define the domains’ positions. In a second step, our ensemble was cross-validated against

experimental double electron-electron resonance (DEER) electron paramagnetic resonance (EPR) measurements. Our two-state ensemble (PDB ID: 7SA5) simultaneously describes the compact and extended states present in solution and shows coupling between intradomain conformation and interdomain positions through rearrangement of hydrophobic residues in the interdomain interface extending to the catalytic pocket⁹. Here, we apply the same method⁹ as summarized in Figure 1 to determine the intra- and interdomain conformational changes due to ligand binding by solving the structure of Pin1 in presence of saturating amounts of pCDC25c and FFpSPR. We obtain ensembles that aid in describing the structural rearrangements upon ligand binding and form the basis of the allosteric mechanisms.

Results And Discussion

pCDC25c, but not FFpSPR, stabilizes the extended state

In agreement with previous reports of Pin1^{2,7}, we observe that upon pCDC25c addition the domain-specific tumbling times (τ_c) of the WW domain decrease relative to those of the PPlase domain (but not FFpSPR). As shown in **Supplemental Table 1**, this indicates decoupling of the two domains and stabilization of extended states. Moreover, we observe between 20 and 26 interdomain NOEs, which have not been reported in previous studies, supporting the partial sampling of compact states.

We utilize DEER to directly measure the distance distribution between the two domains. These EPR measurements involved flash-freezing the samples, allowing for the detection of all distances and their populations at the temperature where the sample vitrifies. Using the double-mutant MTSL-labeled constructs 15-90, 15-98, and 15-131, we measured distances between the two domains for apo and ligand-bound Pin1, while construct 90-131 with both labels in the PPlase domain served as a control (Figure 2a). We performed 4- or 5-pulse DEER measurements and various analyses to detect distances between 15 and 80 Å (**Supplemental Figures 2 and 3**). As expected, the control mutant 90-131 shows narrow distributions that remain nearly unchanged upon ligand addition. While all measurements were fit with unparametrized approaches, constructs 15-90 and 15-98 could also be well described using a bi-Gaussian model (Figure 2a).

Regardless of ligand presence or absence, for constructs 15-90 and 15-98, we see a narrow distance distribution centered around short distances of 22 and 24 Å, respectively, corresponding to a compact conformation (Figure 2a and **b**). A longer, dispersed distance is also sampled and centered around 45 Å, corresponding to the extended state. We have shown previously that apo Pin1 occupies a compact configuration to ~70%⁹. Our measurements show that ligand FFpSPR does not considerably alter this population of the compact state (p_1) as it changes by less than $\pm 5\%$ for both 15-90 and 15-98 constructs compared to apo Pin1 (Figure 2b). Conversely, we see clear proof that pCDC25c stabilizes the extended state: for construct 15-90 the population of the compact state decreases from 68–50%, whereas for 15-98 the compact population only decreases by 7%. We attribute this population discrepancy to the

significant broadening of the extended conformation in the 15-98 construct (Figure 2a, **Supplemental Table 2 and 3**, σ_2 parameters), and the wider rotamer distribution of 98 compared to 90 as previously described⁹, both leading to a higher uncertainty in the population for 15-98. Similarly with PRE (**Supplemental Figure 4**), we see a decrease in R_2^{SP} across domains upon addition of pCDC25c (but not FFpSPR), which also corresponds to an increased distance between the domains. By measuring the enhancement of the transverse relaxation rate (R_2^{SP}) using MTSL-labeled samples, we extracted population-averaged interdomain distances (up to 25 Å) that are used as distance restraints in our subsequent structure calculations.

For the 15-131 construct, the bi-Gaussian model provides a poor description of the distance distribution. For this reason, we are unable to provide a quantitative p_1 value. Nevertheless, we observe two major populations centered around 38 and 43 Å. For apo and FFpSPR-bound Pin1, it appears that the 43 Å peak is populated twice as often as the 38 Å peak, which is a similar ratio (66:33) that we see for the compact and extended populations with the other DEER constructs. This population near 43 Å decreases upon addition of pCDC25c. Due to the correlation of populations between the 15-90 and 15-131 constructs, we believe that the peaks centered around 43 and 38 Å originate from the compact and extended states, respectively. Upon pCDC25c addition, we also see an increase in smaller distances between 20-35 Å and at larger distances around 55 Å that may balance the decrease in intensity around 43 Å. Based on our DEER measurements, we conclude that FFpSPR-binding does not significantly change the apo equilibrium (70:30), while pCDC25c increases the extended population between 7-20%.

Independent assessment of these population shifts comes from slow-exchange NMR peaks observed for residues located in the interdomain interface (**Supplemental Figure 5**)^{3,9,18}. The populations of the compact and extended states from the 15-90 and 15-98 DEER distance distributions are similar to the major and minor slow-exchange peak populations from the N90C (~0.66:0.34) and S98C PRE mutant constructs (~0.78:0.22). Thus, we suggest that these major and minor peaks emanate predominantly from the compact and extended conformations, respectively. This is further supported by the absence of interdomain NOESY peaks for the minor exchange peaks, as expected for extended states. Because the extended state does not contribute appreciably to the interdomain NOEs, we cannot discount the possibility that though the major peaks stems primarily from the compact state also the extended state contributes. Therefore, the slow-exchange minor peaks set a lower limit to the extended population. We note that the relative intensities of these peaks also appear to be sensitive to mutations. In apo and FFpSPR-bound WT Pin1, the average population of the minor peaks is about 10%. Importantly, we consistently see increases in the minor, extended state population upon addition of pCDC25c in both the PRE mutants and WT Pin1 (at least 10% and 5%, respectively). While the populations must be interpreted with caution, we can confidently conclude that the NMR data confirms the DEER data in that pCDC25c, but not FFpSPR, increases the population of the extended state.

Domain positions are mediated by distinct spatial sampling at the interdomain interface

Next, we determined the subtle, intradomain conformational changes that induce the large-scale, interdomain rearrangements. As for apo Pin1⁹, the two-state ligand-bound structural ensembles also produce both compact and extended states (see Methods for details on the structure calculation and **Supplemental Table 5** for structural statistics), which were analyzed to determine correlations between intradomain structure and interdomain organization. In apo Pin1, we detected methyl rearrangement at the PPlase domain's interdomain interface: in the compact state the methyl groups of A140 and L141 point into the interdomain space, while in the extended state these methyl groups point back into the PPlase domain itself⁹. The interdomain interface of the WW domain is also composed of hydrophobic residues, so that this relatively small conformational change (~ 2.5 Å) at the interface likely stabilizes the compact and extended states in apo Pin1 due to a hydrophobic effect. In addition, we detected correlations between the extended and compact states around loops encompassing residues 98-102, 125-128, and 152-154 and the $\alpha 4$ helix⁹.

For the pCDC25c-bound form, we can see a clear difference between extended and compact states on both sides of the interface (encompassing residues 29 and 141 in the graph in Figures 3a, b, and c). Figure 3b illustrates the rearrangement of this interface. As was hinted with the apo Pin1 structure, the compact interface residues point away from their own domain with the ability to make contacts now with the other domain. Conversely, in the extended state these residues point down (T29) or back into the PPlase core (L141), essentially removing the hydrophobic patch from the interface. However, in the FFpSPR-bound form (Figure 5a), this difference between the two states is only maintained in the WW domain's interface (residues 28-30).

As with apo Pin1, in presence of pCDC25c we also observe distinct extended and compact conformations of the $\alpha 4$ helix encompassing residues 131-140 (Figure 3b). This helix is within the interdomain interface, so we expect this conformational difference to also be driven by the presence or absence of the WW domain in the interface. There is also a difference in structure at the PPlase ligand-binding loop residues 125-133 (Figure 3d). These ligand-binding residues are connected to the interdomain interface via the previously mentioned $\alpha 4$ helix. We see a similar RMS deviation between the two states in both apo and pCDC25c-bound ensembles for residues 125-140 (Figure 3e). This not only indicates a mode of "signal" transduction, but also implies how interdomain contact can lead to changes in the ligand-binding site, which in turn control the activity of Pin1. We do not see similar correlations with FFpSPR in this region, likely due to the lack of correlations in the PPlase domain's interdomain interface. FFpSPR binding appears to allosterically disrupt this link between the PPlase interdomain interface and this PPlase ligand-binding loop.

PPlase conformational changes agree with previously proposed allosteric clusters

The aforementioned MD study used an algorithm to link residues into clusters to identify allosteric networks⁴. Apo Pin1 produced two clusters: "path 1" emanating from the canonical interdomain interface and leading to the PPlase β -sheet core, $\alpha 4$, and the loop containing residues 152-154, and "path 2"

starting at the $\alpha 1$ - $\alpha 2$ loop (residues 98-102), $\alpha 2$, and the catalytic loop⁴ as drawn in **Supplemental Figure 6**. Upon addition of FFpSPR, “path 2” extends into the WW domain’s ligand-binding pocket via the bound substrate⁴. The conformational changes we describe upon ligand binding largely agree with these two clusters. While the MD study only examined FFpSPR and the *cis/trans*-locked isosteres, we report that changes due to pCDC25c binding also fall within these clusters which suggests that these allosteric paths are inherent to Pin1.

pCDC25c binding induces intradomain conformational changes

We see evidence of the conformational selection/population shift model of allostery^{19,20} with pCDC25c stabilizing the interdomain extended state as well as a few regions of the correlated intradomain extended state. However, we also observe much larger conformational changes throughout Pin1. Figures 4a and c compare the two-state ensembles of apo and pCDC25c-bound Pin1 from various vantage-points, and residue-specific RMS deviations between the two forms are plotted in Figure 4b. In the following, we primarily discuss key conformational changes between the apo and pCDC25c-bound structures. Whenever necessary, we additionally focus on differences between the compact and extended states within each ensemble.

In the WW domain (Figure 4a), the ligand-binding loop (residues 15-22) folds upon pCDC25c binding with a RMSD of up to 8 Å. Previous crystal structures have shown that ligands bind on “top” (in our view) of this loop and within the pocket^{11,17}. This increased compaction within the WW domain upon pCDC25c binding was also reported in a recent study using PRE and MD simulations¹⁵. The presence of the ligand and the conformational change in this loop connecting $\beta 1$ and $\beta 2$ may perturb residues 34-36. In absence of ligand, the side chain of E35 points toward the ligand-binding loop, but binding of pCDC25c causes the side chain to point up to 4 Å away from the unbound position. Residues 34-36 appear to act as a hinge point that causes the $\beta 3$ strand to tilt, which ultimately also changes the interdomain interface itself. The methyl group of T29 points directly into the interface (or into the PPlase itself) without ligand present, while the methyl group is more excluded from the interface (by pointing “downward”) when pCDC25c is present. The degree of methyl occlusion from the interface with pCDC25c is dependent on the compact and extended states of the two-state ensemble. In interface residue T29, the compact state in presence of pCDC25c is more similar (but still somewhat pointed down relatively) to either state of apo residues, while the methyl group in the extended state moved 3 Å away and is occluded from the interface. In summary, our WW structures show (i) the ligand-binding loop “folds” upon binding pCDC25c, (ii) this perturbation shifts residues 34-36 and therefore the $\beta 3$ strand, which ultimately (iii) reorients the interdomain interface.

In the PPlase domain’s interdomain interface (Figure 4c), we see the compact state methyl group of L141 pointing more into the interface than the extended state for both apo and pCDC25c-bound Pin1. The position of the L141 side chain is a “gradient”: the apo compact state points farthest into the interface, followed by the nearly overlapping apo extended state and pCDC25c-bound compact state and lastly, the

pCDC25c-bound extended-state side chain is essentially buried in the PPlase. The PPlase interdomain interface is located near and within the C-termini of the $\alpha 4$ helix. Upon pCDC25c binding, this helix displays small changes up until its N-termini (i.e. residue P133), and conformational changes also occur in residues 128-131 that make up part of a loop responsible for ligand-binding in the PPlase. Above, we also pointed out clear correlated differences throughout this helix and ligand-binding loop between the compact and extended state of pCDC25c-bound Pin1 (Figure 3), highlighting distinct motions induced by WW contact.

Compared to the apo form, there is also a shift in the entire bound-form core β -sheet propagating from nearby the interdomain interface $\beta 6$, passing through $\beta 7$, $\beta 4$, and finally $\beta 5$. Residues located in these core β -strands are critical for ligand binding and catalysis in the PPlase and the observed conformational changes are located in the “path 1” cluster previously proposed by MD simulations⁴. In addition, we also see changes in the loop encompassing residues 152-154 that are critical for catalysis^{17,21}. The two-state structure of apo Pin1 showed that this loop position is correlated with interdomain contact⁹. After pCDC25c-binding, the correlation disappears and the loop position in both states adopts the apo extended-state position. This is evidence for the conformational selection model of allostery^{19,20,22,23}, where multiple conformations preexist but the populations shift upon ligand binding.

In agreement with the “path 2” allosteric cluster, the reorientation of the $\alpha 1$ helix propagates conformational changes to the $\alpha 2$ helix and the catalytic loop. The C-terminus of the long $\alpha 1$ helix appears to interact with the WW domain in the compact state. In apo Pin1, K97 interacts with the WW domain around residues 32-33. Upon pCDC25c-binding, the WW domain reorients away from the $\alpha 1$ helix, and the side chain of K97 moves in the opposite direction as the WW domain abolishing any contact between the two domains in this region. The N-terminus of the $\alpha 1$ helix is connected to the PPlase catalytic loop (residues 65-82), which also is slightly repositioned after binding to pCDC25c.

Although we see conformational changes that appear to propagate from the interdomain interface to the PPlase catalytic site, we are unable to ultimately conclude that the changes are due to the contact (or lack-there-of) with the WW domain as the PPlase itself binds/isomerizes pCDC25c as well. We note, however that the binding affinity is at least one order of magnitude lower than for the WW domain and the CSPs are also relatively weak in the active site upon ligand binding²⁴. Until the structural ensemble of the isolated PPlase is solved with pCDC25c bound (no solution structure of the isolated PPlase bound to any ligand has been deposited in the PDB to date), we can only hypothesize which perturbations link the WW domain to the PPlase catalytic site. In summary, regions near the active site that undergo a conformational change and also exhibit translational connections to the interdomain interface involve firstly the core β -sheet and C-terminal ligand-binding region (residues 128-131) from “path 1” and secondly the catalytic loop including residues 68 and 69 from “path 2”. The latter is responsible for binding the ligand’s phosphate.

FFpSPR induces different conformations than pCDC25c

In contrast to pCDC25c-binding, the distance distributions determined from DEER measurements demonstrate that no major interdomain rearrangement occurs in presence of ligand FFpSPR. Our two-state structural ensemble of FFpSPR-bound Pin1 (Figure 5a) also reveals distinct intradomain conformational changes that differ from the pCDC25c-bound ensemble (Figure 6b).

First of all, we see similarities between the ligand-bound structural ensembles around residues 34-36, 112-113, and 152-154 (Figure 6b, Figure 6c **and e**). As examined previously with pCDC25c, the conformational change around residues 34-36 may have reoriented the β 3 strand to cause an alteration in the WW domain's interdomain interface. When FFpSPR is present, this region shows a change in spatial sampling: while the compact state is in closer agreement with the apo conformation, the extended state is more similar to the pCDC25c conformation (Figure 5b). The other similar conformational changes occur in the PPlase catalytic site, and these conformational changes may be conserved for efficient ligand catalysis (Figure 6e).

Secondly, we see many changes in Pin1's structure dependent on the ligand. Starting with the WW domain: the ligand-binding loop folds upward upon binding to pCDC25c, but it moves in the opposite direction with FFpSPR (Figure 6a-c). The arrangement of the WW domain's interdomain interface is also dependent on ligand sequence, as key residue I28 moves down and diagonally with FFpSPR (Figure 5b), while pCDC25c only causes the side chain to move down (Figure 6c). Additionally, in presence of pCDC25c the side chains of I28 and T29 are more blocked from the interface than with FFpSPR. Comparing the compact state ensembles at the interdomain interface (Figure 6d), it is apparent that pCDC25c occludes the methyl group of T29 more than FFpSPR, whereas upon FFpSPR-binding the methyl group of A31 points more towards the PPlase domain than in the apo and pCDC25c-bound compact states. Overall, the distinct rearrangements of the WW interdomain interface likely cause the disparate changes in the population of the compact and extended states.

In the PPlase domain's interdomain interface, FFpSPR eliminates the correlation between the two states (Figure 5A) with both states sampling an intermediate conformation similar to the apo extended state and the pCDC25c-bound compact state (Figure 5c, Figure 6e). FFpSPR does not increase the stability of the interdomain extended state. However, this ligand still appears to stabilize the conformation at the interdomain interface (if not further occlude the methyl groups), and subsequently stabilizes the extended conformation at the β 6- β 7 (152-154) loop (Figure 4C, Figure 5e, Figure 6e). Both ligands also cause a change in the residues (125-130, β 5, and β 5- α 4 loop) that are responsible for binding to the ligand C-terminal of the pS/T-P motif, yet pCDC25c causes a larger RMSD with respect to the apo state in this region (up to 7 Å vs. 3.5 Å). Unlike pCDC25c, FFpSPR does not cause a large-scale shift to the β -sheet core.

Within "path 2", we see a major change in the orientation of the α 1 helix and the subsequent rearrangement of the PPlase catalytic loop (residues 65-80) upon addition of FFpSPR (Figures 5e and 6a). FFpSPR causes more expansive changes in the PPlase catalytic loop than pCDC25c (Figures 4C and 6a) even though with pCDC25c we observe larger chemical shift perturbation in ^{15}N -HSQC ligand

titrations (**Supplemental Figure 1c**). The catalytic loop position is moderately similar between apo and pCDC25c-bound Pin1 except for the region surrounding residues R68/69, while with FFpSPR the catalytic loop shifts in the same direction as the N-terminus of the $\alpha 1$ helix (Figure 6e). Within this hypothesized “path 2”, pCDC25c- but not FFpSPR-binding leads to reorientation of the $\alpha 2$ helix that is C-terminal to the $\alpha 1$ helix (Figure 4C). Therefore, the ligands differ in their impact on “path 2”: whereas FFpSPR has a greater effect on the catalytic loop, pCDC25c causes a greater change in the $\alpha 2$ helix.

FFpSPR-binding also leads the $\beta 5$ strand and $\beta 5$ - $\alpha 4$ loop around residue 125 to move away from the rest of the PPlase (Figures 5e, 6a and 6e). This strand contributes to “path 1”, yet no clear additional changes propagate from the interdomain interface to this region. As this area is a part of the ligand-binding site in the PPlase, we expect that FFpSPR binding directly alters this spot instead of an allosteric effect.

Summary of ligand-specific conformational changes within the allosteric network

Ligands FFpSPR and pCDC25c induce an antagonistic conformational change in the WW-binding loop that leads to a disparate change in the interdomain interface likely provoked by residues 34-36 and through the $\beta 3$ strand. Residues 28-29 are further occluded from the interface with pCDC25c, while in presence of FFpSPR these residues are further reorganized (but less buried than pCDC25c). Subsequently, the PPlase interdomain residues are also further occluded with pCDC25c, while the residues adopt an intermediate position with FFpSPR. The pCDC25c-bound structure shows large conformational changes that originate from the interdomain interface and propagate to the catalytic site through the previously proposed “path 1”⁴. While FFpSPR also causes some changes through the interdomain interface and “path 1”, our structures show larger conformational changes propagating from the $\alpha 1$ helix into the catalytic loop in “path 2”. Conversely, pCDC25c does not cause as substantial change through the $\alpha 1$ helix. We speculate that the different interdomain distance present in the two pathways determines the observed severity of the conformational change. FFpSPR does not alter the interdomain distance which allows the ligand to interact through the $\alpha 1$ helix when bound to the WW domain. On the contrary, pCDC25c stabilizes the extended state, greatly reducing the ability of the ligand bound to the WW domain to also interact with the $\alpha 1$ helix. One major similarity is that Pin1 binding to either of these ligands stabilizes the apo extended state structure in the PPlase interdomain interface (residues 140-141) and the $\beta 6$ - $\beta 7$ loop (residues 152-154), supporting a conserved mode of conformational selection.

Conclusion

Inter- and intradomain spatial sampling may be coupled in many multi-domain proteins. We have used our recently introduced NMR- and EPR-based approach to demonstrate such coupling in the two-domain protein Pin1, which rationalizes ligand-specific interdomain allostery. Specifically, FFpSPR binding does not change Pin1’s interdomain equilibrium while pCDC25c stabilizes the extended state. We have shown that the conformation of the hydrophobic residues in the interface controls the population of the extended and compact states. Our structural ensembles demonstrate that pCDC25c and FFpSPR cause

distinct conformational changes in Pin1 primarily through previously proposed “path 1” and “path 2”, respectively. Ligand FFpSPR leads to a large displacement of the PPlase catalytic loop with essential residues 68-69 responsible for phosphate-binding, but pCDC25c features critical changes throughout the core β -sheet that could rearrange the active site residues. Overall, our ensembles demonstrate that pCDC25c stabilizes the inter- and intradomain extended state, whereas FFpSPR causes distinct conformational changes while maintaining the interdomain equilibrium.

Methods

Materials

The protein expression and purification, the measurements of NOE buildups, scalar couplings, structure calculations, relaxation rates, PRE, and DEER experiments were extensively described in our recent publication on apo Pin1⁹.

Ligands FFpSPR and pCDC25c (sequence EQPLpTPVTDL) were synthesized by the Peptide and Protein Chemistry core at Univ. of Colorado Anschutz using Fmoc solid-phase synthesis. The N-termini were acetylated while the C-termini were amidylated to protect the peptides against degradation. HPLC was performed to purify the peptides, and samples were analyzed by LC/MS which confirmed purity >96%. The samples were lyophilized and resuspended in NMR buffer (20 mM sodium phosphate, 50 mM sodium chloride, 5 mM dithiothreitol, 0.03% sodium azide at pH 6.5) dependent on their solubility. Ligand pCDC25c was more soluble (40 mM stock) than FFpSPR (4 mM stock) in this buffer, likely due to the hydrophobic phenylalanines in the latter. No DMSO was used.

NMR Spectroscopy

For assignment, scalar coupling, relaxation, and NOESY experiments, all samples contained ^{15}N , ^{13}C -labeled Pin1 in NMR buffer at pH 6.5 with 3% D_2O (low D_2O to reduce H-D exchange). While the apo sample contained ~2 mM Pin1, the amount of protein had to be reduced for the ligand-bound samples due to low concentration of FFpSPR, and to reduce aggregation with pCDC25c. Therefore, the FFpSPR-saturated sample contained 600 μM Pin1 and 3.6 mM FFpSPR and the pCDC25c-saturated sample contained 750 μM Pin1 and 4 mM pCDC25c. Apo Pin1 was assigned as described previously⁹. Using ^{15}N -HSQC, ^{13}C -resolved aliphatic and aromatic CT-HSQC, and a $^{15}\text{N}/^{13}\text{C}$ -resolved $[\text{}^1\text{H}-\text{}^1\text{H}]$ NOESY experiment, we assigned the chemical shifts of the FFpSPR- and pCDC25c-bound Pin1 based on the apo Pin1 assignment¹⁸. While chemical shift changes occur, the NOESY towers remain relatively intact and characteristic of each atom. The chemical shifts of FFpSPR-bound and pCDC25c-bound Pin1 have been deposited in the BMRB under accession codes 51043 and 51034, respectively. NOE buildup series were run as previously described¹ with mixing times of 24, 32, 40, 48, and 56 ms. Scalar coupling ($^3J_{\text{HN-H}\alpha}$, $^3J_{\text{H}\alpha\text{-H}\beta}$ ²⁵, and $^3J_{\text{N-C}\gamma}$) and relaxation (R_1 and $R_{1\rho}$ for determination of tumbling times) experiments were also recorded on these ligand-bound samples as previously described⁹.

The same cysteine-mutant constructs for PRE and DEER that were developed to measure the interdomain orientation of apo Pin1⁹ were also used for ligand-bound measurements. The samples were expressed, purified, and spin-labeled as previously published⁹. For PRE, the ligands were added to be 8x the concentration of Pin1. R_2 PRE rates were measured on the ¹⁵N-labeled MTSL-conjugated (paramagnetic) and quenched (diamagnetic) samples M15C, N90C, S98C, and Q131C that all also contained C57A and C113D mutations. The relaxation enhancement due to the paramagnetic labels (R_2^{sp}) were obtained from the R_2 difference between the paramagnetic and diamagnetic samples²⁶. The DEER samples M15C-N90C, M15C-S98C, M15C-Q131C, and N90C-Q131C were all measured with 8x ligand.

Data fitting and analysis for the ligand-bound samples were performed as previously published⁹ for apo Pin1.

Pulsed EPR spectroscopy: Double Electron-Electron Resonance

Pulsed Double Electron-Electron Resonance (DEER) Q-band EPR measurements were recorded at 50 K with a Bruker Eleksys E580 spectrometer equipped with a home-built resonator²⁷ and an incoherent arbitrary waveform generator pulse channel. 40 μ L of 30-80 μ M MTSL-labeled Pin1 in buffer:glycerol-d₈ 1:1 v:v were filled in 3 mm o.d. quartz capillaries, flash frozen and stored in liquid nitrogen between measurements. Echo-detected fieldsweeps were recorded using $\pi/2 - \tau - \pi - \tau$ with pulse lengths $t_{\pi/2} = t_{\tau/2} = 12$ ns and an interpulse delay of $\tau = 400$ ns. DEER-EPR data were acquired either with the 4-pulse DEER (4pDEER) sequence $\pi/2_{obs} - \tau_1 - \pi_{obs} - (\tau_1 + t) - \pi_{pump} - (\tau_2 - t) - \pi_{obs} - \tau_2$ ²⁸ or the 5-pulse DEER (5pDEER) sequence $\pi/2_{obs} - (\tau/2 - t_0) - \pi_{pump} - t_0 - \pi_{obs} - t - \pi_{pump} - (\tau - t + \delta) - \pi_{obs} - (\tau/2 + \delta)$ ²⁹ featuring a time shift $\delta = 120$ ns to separate the refocused from the stimulated echo³⁰. 4pDEER measurements were performed with monochromatic, rectangular pulses of length $t_{\tau,pump} = 12$ ns, applied at the maximum of the nitroxide Q-band spectrum and observer pulses with $t_{\tau/2,obs} = t_{\tau,obs} = 16$ ns, offset 100 MHz from the pump frequency. 5pDEER measurements were recorded with HS{1,6} pump pulses of 150 MHz width and monochromatic, rectangular observer pulses with $t_{\tau/2,obs} = t_{\tau,obs} = 32$ ns, placed 70 MHz away from the pump position. In each case, nuclear modulations were averaged using an eight-step phase cycle with 16 ns steps.

All DEER data were analyzed using an earlier version of DeerLab (DL) based on Matlab³¹ (release 0.9.2, available under <https://github.com/JeschkeLab/DeerLab-Matlab>) by modelling the background decay by a stretched exponential function (bg_stexp , $B(t) = \exp(-\kappa|t|^d)$) with the decay rate κ and the stretch factor d restraint to 0.02-1 μs^{-1} and 0.9-1.2, respectively. 4pDEER and artefact corrected 5pDEER data³² were analyzed using the single-pathway 4pDEER experiment model (ex_4pdeer) including the modulation depth λ as a fit parameter. For the analysis of the primary 5pDEER data, the multi-pathway 5pDEER model (ex_5pdeer) was applied. This model consists of an unmodulated pathway, the 5pDEER pathway and 4pDEER pathway ("artefact" that refocused at time T_0 ⁽²⁾) with amplitudes Λ_0 , λ_1 and λ_2 , respectively.

Parameter-free and Gaussian distance distributions $P(r)$ were computed via Tikhonov regularization, using generalized cross-validation (GCV) to select the optimal regularization parameter. Bootstrapping with 200 samples produced converged 95% confidence intervals (CI), listed in **Supplemental Tables 2-4** for all fitting parameters. Neural network analysis was performed by DEERNet SVN rev 5662, using the Comparative DEER Analyzer feature of DeerAnalysis 2021.

Multi-state structure calculations

As with apo Pin1 (PDB ID: 7SA5), we combined all PRE, eNOE, and scalar coupling restraints and calculated multi-state ensembles of ligand-bound Pin1. To account for the time- and ensemble-averaged nature of the probes, these restraints must be fulfilled by the average of the back-calculated contributions from each individual state. To allow large spatial sampling between the two domains, we 1) calculated the structure of the WW domain and then 2) froze the WW angles and used them as an input to determine the PPlase structure and interdomain positions⁹. The PDB IDs for FFpSPR- and pCDC25c-bound two-state ensembles are 7SUQ and 7SUR, respectively.

Similar to our multi-state ensemble calculations of apo Pin1, the CYANA target function (TF, proportional to the sum of squared violations) was high for the single state structures ($\sim 300 \text{ \AA}^2$ for all three ensembles) but decreased significantly upon addition of a second state to fulfill all the structure restraints (**Supplemental Table 5**). Note that the single-state structure is more akin to an averaged structure. All three Pin1 single-state structures resulted in a compact conformation as all the interdomain NOEs needed to be fulfilled. When a second state was allowed, an extended and a compact state were generated with all the interdomain NOEs fulfilled in the compact state.

While the interdomain NOEs and PREs were sufficient in orienting the two domains in apo Pin1 to agree with the DEER distribution, the position of the extended states did not fulfill longer distances from the DEER distance distributions with the ligand-bound ensembles. Therefore, we calculated population- and fluctuation-averaged distances between the C β s of the spin-labeled residues based on the DEER distance distributions and used them as restraints in our structure calculations. A tolerance of $\pm 5 \text{ \AA}$ was added to these effective distances to use as upper and lower limit restraints. While we optimized and lowered the PRE distance restraint weight to 0.01 (relative to the NOE weight of 1)⁹, we kept a weight of 1 for these DEER effective distance restraints as there were only 3 distances added for the calculation, and these restraints did not appreciably increase the target function. Importantly, the addition of the DEER effective distances adjusted the interdomain positions but did not change the intradomain structural correlations. When we calculated a ligand-bound two-state ensemble with the interdomain NOE, PRE, and averaged DEER restraints (see Supplemental Information), we obtained a satisfactory match to the experimental DEER distributions, with only a slight under-representation of small populations of longer distances.

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Figures

Figure 1

Schematic of conformational changes due to ligand binding in Pin1 and experimental methods used to detect them.

Figure 2

Impact of ligand binding on the DEER distance distributions of Pin1. a) DEER distributions are plotted including 95% confidence intervals for each construct obtained from either a DEERNet neural network analysis or bi-Gaussian fit. MTSL positions (pink) and distances (black) are drawn onto the WW (orange) and PPlase (blue) domains of the x-ray structure 1pin17. b) Gaussian parameters for 15-90 and 15-98 constructs, where r_i = distance, σ_i = distance standard deviation and p_i = population with index $i = 1$ and $i = 2$ corresponding to the compact and extended state, respectively.

Figure 3

Coupling of inter- and intradomain spatial sampling of pCDC25c-bound Pin1. The two-state ensemble is analyzed with the states sorted by presence or absence of interdomain contact (compact or extended state, respectively). Major RMS deviations between the two states of pCDC25c-bound Pin1 are shown in a) WW domain, b) interdomain interface, c) PPlase domain side with interdomain interface, and d) PPlase domain's catalytic site. e) RMSD between mean compact and extended states of domains is plotted versus residue number with error bars depicting standard deviation of conformers. The RMSD versus residue number of the apo Pin1 two-state ensemble are overlaid in the graph. The interdomain interface is highlighted green in the graph.

Figure 5

Conformational changes of Pin1 upon binding FFpSPR. a) RMS deviation between mean compact and extended states of FFpSPR-bound Pin1 is plotted versus residue number with error bars depicting standard deviation of conformers. Corresponding RMSD values of the apo Pin1 two-state ensemble (PDB ID: 7SA5) are overlaid (yellow line). The interdomain interface is shaded in green. Regions where both pCDC25c and FFpSPR caused similar conformational changes are colored in light grey. b) WW and c) PPlase domains of the two-state structural ensembles of FFpSPR-bound and apo Pin1 are overlaid. Key conformational changes are shown in the structure and on the graphs. Residues of the interdomain interface shown are colored green. d) Compact state of apo and FFpSPR-bound Pin1.

Figure 6

Comparison of two-state structures of apo, pCDC25c-bound, and FFpSPR-bound Pin1. a) RMS deviation between the mean structures is plotted versus residue number. Key residues are noted, and interdomain interface is shaded green. b) Difference between RMS deviations of FFpSPR from apo and from pCDC25c. Regions with structure more similar to the pCDC25c-bound structure have positive values, and regions more similar to the apo structure have negative values. c) Superposition of mean two-state

structures of the WW domain in apo and bound forms. d) Comparison of the compact-state interdomain interface. e) Superposition of mean two-state structures of the PPlase domain.

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