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Review

Dynamical binding of proline-rich peptides to their recognition domains

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Abstract

Recognition of proline-rich sequences plays an important role for the assembly of multi-protein complexes during the course of eukaryotic signal transduction and is mediated by a set of protein folds that share characteristic features. For many complex systems containing proline-rich sequences, multiple binding modes have been found by theoretical and/or experimental studies. In this review, we discuss the different binding modes as well as the correlated dynamics of the peptides and their recognition domains, and some implications to their biological functions. Furthermore, we give an outlook of the systems in the context of systems biology.

Keywords: PRS (proline-rich sequence); SH3 domain; GYF domain; WW domain; Register shift; Peptide binding

1. Introduction

Intracellular protein domains recognizing proline-rich sequences (PRS) play a pivotal role in biological processes that require the coordinated assembly of multi-protein complexes [1]. One example is Src kinase, where the terminal SH3 domain recognizes a long proline-rich stretch linking the nearby SH2 domain with the catalytic kinase domain [2]. In vertebrate genomes, PRS are predicted to be among the most abundantly expressed amino acid sequence motifs [3] and this corresponds to an increasing number of proteins that acquired PRS-recognition domains during the course of evolution [4]. Up to now, the super-family of proline-rich sequence recognition domains consists of profilin [5], the SH3 [6,7], the WW [8], the EVH1 [9], the GYF [10,11], the UEV [12,13] and probably the ligand binding domain of prolyl-4-hydroxylase [14]. For each of these domains, a set of conserved aromatic amino acid residues is important for peptide binding (see Fig. 1).

The PRS and their recognition domains as well as the common structure function relationships have been recently reviewed several times [4,15–17]. Here, we will focus on the dynamics and conformational variability for both interaction partners, the roles of these changes in the binding process as well as their potential biological advantages. At the end of this mini-review, we take a look into the future and point out fruitful areas, e.g., in systems biology, for further studies.

2. Proline and proline-rich sequences

Among the 20 naturally occurring amino acids, proline is the only one in which the side chain atoms form a pyrrolidine ring with the backbone atoms (see Fig. 2a). This cyclic structure leads to some distinguished properties of proline: it induces conformational constraints among the atoms in the pyrrolidine ring, and it is the reason for the slow isomerization between cis/trans conformations [18] and for the secondary structure preferences of proline-rich sequences (see below). Remarkably, the cis/trans preference of proline-X (where X is any amino acid) peptide bonds are different in different solvent environments [19–23].

Due to the special properties of the proline residue, the proline-rich sequences tend to form either of two different secondary structures: PPI helices (in which all prolines are cis isomers) and PPII helices (where all prolines are trans isomers)(see Fig. 2b and Table 1). The PPII helix is a left-handed helix with three residues per turn (see Figs. 2b and c). It has a three-fold symmetry when viewed along the helical axis

Abbreviations: PRS, proline-rich sequences; SH2/SH3, Src homology 2/3; GYF, glycine-tyrosine-phenylalanine; WW, protein domains containing two conserved tryptophans (W) spaced 20–22 amino acids apart; PPI/PPII, poly-proline helix type I/II

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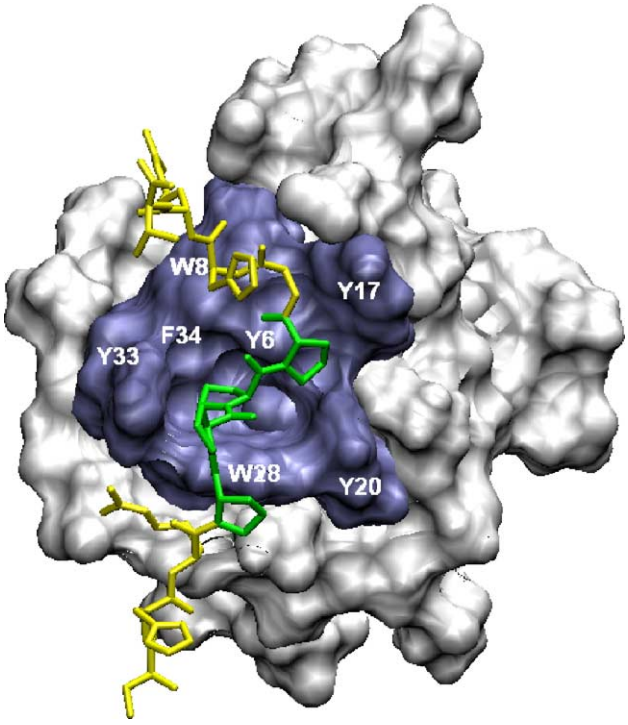


Fig. 1. NMR structure of GYF domain with wild-type peptide [72]. The GYF domain is represented by its molecular surface; the peptide atoms are drawn as sticks. Residues forming the binding pocket are colored in blue and labelled by their one-letter codes and sequence numbers. The four proline residues are colored in green.

and every forth residue is in the same position (at a distance of 9.3 Å from each other). Along the same axis, the PPII helix also has a two-fold rotational pseudo symmetry [4]. The side chains and backbone carbonyl groups are located in similar positions in both orientations along the backbone axis. This leads to the special property that, e.g., SH3 domains may bind their PRS ligands in two orientations [24]. Due to the lack of intramolecular hydrogen bonds, PPII helices are more flexible than α-helices [25] and the backbone groups are more accessible to the solvents. This also means that the PPII motifs are mostly located on the surface of proteins [26]. All these geometric features are important when PPII helices bind to the recognition domains. Switching between cis and trans forms occurs either spontaneously and slow [18] or is catalyzed by cis/trans isomerases as cyclophilins (Cyp), FK506-binding proteins (FKBPs), and the parvulins [27,28].

Rucker and Creamer argued that PPII is an energetically favorable option for oligopeptides because all backbone polar groups are well solvated in this conformation in water, thus compensating for the lack of intramolecular hydrogen bonds [29]. Theoretical studies argued that PPII helices disrupt water organization less than β-sheet and α-helices, which makes them entropically favored as well [30,31]. However, the dynamical features of PRS were emphasized by Scheraga and coworkers. They claimed that viewing the optimal conformation of a polyproline-rich peptide as an ideal or canonical PPII helix in water is an oversimplification, and one should consider *cis–trans* isomerization of the proline peptide groups [23]. In contrast to the aqueous environment, where either PPI or PPII seems possible, all PRS peptides known so far adopt a pure polyproline type II helix upon binding to the recognition domains [6,7,10,11,32]. For peptides only containing few prolines, the cis isomer is favorable as well [33–35] and the *cis–trans* isomerization in these scenarios is often connected to the functions of the proteins [33,35]. An impressive example for such conformational control is a proline-driven conformational switch within the Itk SH2 domain [35]. Two structures of Itk SH2 determined by NMR spectroscopy corresponding to the cis and trans imide bond-containing conformers indicate that the heterogeneous Pro residue acts as a hinge modulating ligand recognition by controlling the relative orientation of protein-binding surfaces. Therefore, *cis–trans* isomerization of a single prolyl imide bond within the SH2 domain mediates conformer-specific ligand recognition. This plays a functional role in mediating distinct intermolecular interactions with exogenous signaling partners, e.g., cyclophilin A (CypA), and further influencing the T cell activation [35,36].

3. Preformation of the PPII helix for unbound PRS

It has been recently recognized that many proteins contain long disordered segments in their functional states under physiological conditions [37–41]. For example, most of the polypeptide hormones are conformationally disordered in aqueous solution and fold upon binding to their receptors [40]. Such unstructured segments within large proteins provide ideal scaffolds for the interaction with several different targets and thereby help to assemble multi-protein complexes [37–41]. For those proteins with unstructured segments, the coupling of binding and folding is expected favorable in terms of the binding free energy: the entropic penalty associated with the folding transition is counterbalanced by a large enthalpy of binding [37,42]. In those cases, the folding upon binding acts as a fine controller of the thermodynamic balance.

On the other hand, it has been shown by many experimental and theoretical studies that certain peptides, including proline-rich sequences, adopt preferred conformations in solution [1,23,29,43–51]. Therefore, it is a matter of ongoing discussion whether the PPII helix is such a preferred conformation for particular peptide sequences [13,29–31,43,46,48,49,52–57]. A mechanistic description of the binding event has to distinguish whether the PPII helix conformation is preformed in the unbound peptides and binding to the recognition domains takes place in a “lock and key” mode or whether folding and binding occur in parallel, corresponding to an ‘induced fit’ model (see Scheme 1).

We have previously studied the binding of wild type and some mutated PRS binding to the GYF adaptor domain by a combined theoretical (molecular dynamics simulations) and experimental (NMR and phage display) approach [58]. The polyproline peptides considered in this study were found to be already folded into a PPII helix conformation in the unbound state and bind constitutively to the GYF domain. Obviously, this

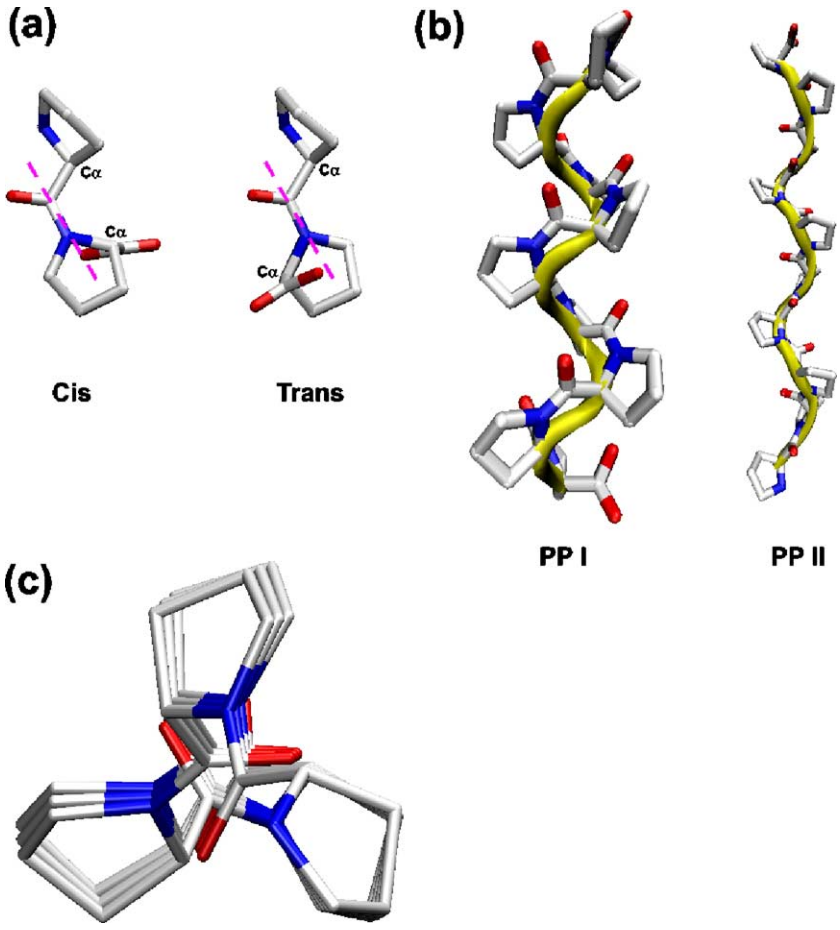


Fig. 2. Structure of (a) the cis and trans proline residues; (b) the PPI and PPII helices; (c) the PPII helix viewed along the helical axis. Molecules are shown as sticks. Oxygen and nitrogen atoms are colored in red and blue, respectively.

binding scenario is entropically more favorable than binding of unstructured peptides. The stiff PPII helix conformation of the unbound peptides studied is apparently intrinsically stable in solution and is also favorable for its specific binding motif. An experimental study addressed the binding of the polyproline Sos peptide to the Sem-5 SH3 domain [43]. They found that the PPII bias of unstructured peptides is driven by a favorable and significant enthalpy (ΔH) of -1.7 kcal mol^{-1} residue^{-1}, which is partially offset by an unfavorable entropy ($T\Delta S$) of -0.7 kcal mol^{-1} residue^{-1}, relative to the ensemble of disordered conformations of the molecule. A similar example is the c-Myb oncoprotein, which adopts an α-helical conformation both complexed and uncomplexed with its target protein [42]. Remarkably, binding of c-Myb to its target (residue 586–672 of CREB binding protein) is entropically favored ($\Delta S = +7.5$ cal mol^{-1} K^{-1}) while its favorable enthalpy change is small ($\Delta H = -4.1$ kcal mol^{-1} K^{-1}) [37,42].

Table 1
Geometric properties of polyproline helices I and II

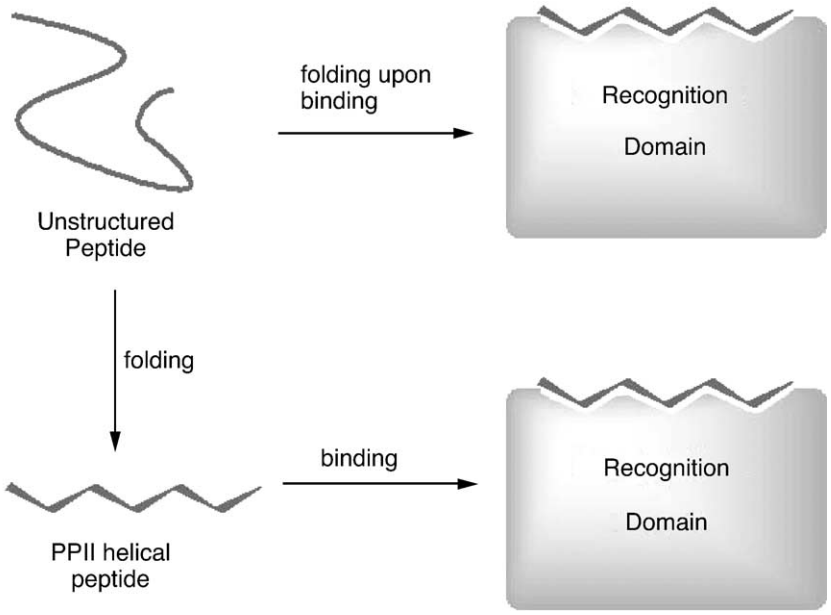
Type of helix	Phi (°)	Psi (°)	Omega (°)	Number of residues per turn	Helical rise per residue (Å)	Helical pitch (Å/turn)
PPI[a]	−75	160	0	3.3	1.7	5.6
PPII[b]	−75	145	180	3.0	3.1	9.3

[a] PPI helix is right-handed.
[b] PPII helix is left-handed.

In conclusion, it appears that the conformation of unbound peptides may be fine-tuned for a particular functional range of peptide binding. On the one hand, Dyson and Wright proposed that unstructured proteins provide a large flexibility of binding reactions because they may adopt various structures upon binding to different partners [40]. On the other hand, as exemplified here for the GYF domain–ligand pair, the preformation of a peptide conformation might be well suited to guarantee the rapid formation of specific peptide–protein complexes within the dynamic settings of signal transduction.

4. Different binding modes and their roles for binding and function

A classic NMR study [24] showed that SH3 domains can bind proline rich ligands in two orientations, due to the two-fold rotational pseudo symmetry of the PPII helix along the helical axis (as discussed in the first part of this review). Is such a scenario possible for other domains as well and what is the biological advantage of these different binding modes? Newer crystallographic evidence then showed that profilin, like SH3 domains, can bind proline-rich peptides in two distinct amide backbone orientations [59]. As has been previously proposed



Scheme 1. The PRS peptide may either adopt its PPII-conformation while binding to the recognition domain (top) or it may transiently or permanently adopt a PPII-helical conformation in solution and bind to the recognition domain in its folded conformation (bottom).

for SH3-related functions, the ability of profilin to bind ligands in multiple orientations may control the organization of multi component signaling complexes, and provides a mechanism for the regulation of actin cytoskeleton assembly.

How far does this conformational flexibility of recognition extend? In some cases, as for the Itk SH2 domain, a single proline flip may result in an on/off control of binding events. On the other hand, Piotukh et al. studied linear peptide motifs binding to CypA using both experimental (phage display, NMR) and theoretical (docking and molecular dynamics simulation) approaches. They predicted that the peptides, which contain proline residue in the binding motifs, can bind to CypA with both cis and trans prolines maintaining similar interactions between the peptides and CypA [34].

In the study of PRS peptides with GYF domain, a register shift motion of the peptides was found for wild type and mutated complexes [58] (see Fig. 3): Pro5 and Pro6 of the peptide inserted into the binding pocket instead of Pro6 and Pro7 in the original binding modes. Although all four prolines in the peptide are rotated clockwise when viewed from the C to the N terminus, interestingly, the orientations of the remaining residues were kept and showed only a slight translation toward the C terminus.

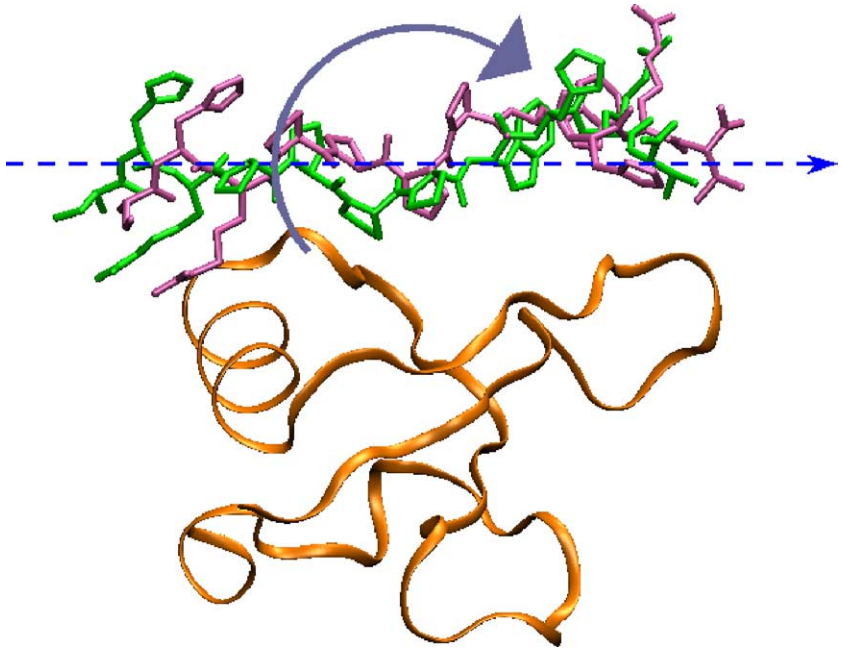


Fig. 3. The translation and rotation motions of the peptide between the two binding modes of the PRS ligand (shown as sticks) binding to the GYF domain (shown as ribbons).

Therefore, all interactions between the peptides and the domain (e.g., electrostatic attractions, hydrophobic interactions and intermolecular hydrogen bonds) were kept. This observation indicates an additional alternative binding mode of the peptides due to their three-fold symmetry around the helical axis (rather than the two-fold rotational symmetry along the helical axis).

What is the function of these different binding modes? Providing two alternative binding modes for a peptide should, theoretically, provide a small additional stability for the bound conformation due to the larger number of states accessible inside the minimum energy well of the bound state. Therefore, this 'screw-like' rotation-translation motion or the transition between different binding modes can decrease the entropic penalty of the binding without affecting the specificity. For the "shift in register" transition between different binding modes of the PRS–GYF system, we suggested an additional function that is related to the binding mechanism: the peptides may bind or leave the binding interface on the recognition domain by this "screw like" motion along the interface. Such screw-like motions may allow for a kinetically favorable binding process by "stripping off water molecules" upon binding and/or unbinding. Furthermore, these sites might act as delocalized anchors within protein associations that rely on fast structural rearrangements within the context of eukaryotic signal transduction [58].

5. Conclusions and perspectives in systems biology

Proline-rich sequences and their recognition domains are of particular biological importance in signal transduction and complex assembling. The special geometric and chemical properties of proline and PRS make the binding of PRS to the recognition domains rapid and week. In the systems biology point of view, these systems have important roles as mediators in the protein–protein interaction networks of cells (see below). However, recent evidence supports the notion that these interactions are not simple on/off reactions but may be fine-tuned and/or regulated by delicate conformational transitions.

Currently, one of the greatest challenges facing cellular proteomics is to understand the roles of thousands of proteins acting as principal components of a cell, and how they interact to create this complex but organized "machine". This network of interactions, also termed the "interactome", is only one part of the cellular network that also includes the gene regulation network, the metabolic network, the functional network and so on [60–66]. We argue that for protein–protein interaction networks, three-dimensional structural information is essential for the correct and meaningful establishment of the networks [67]. The reason is that many proteins interact with each other via extended surfaces composed of 10–30 residues that may be far apart in the sequences. In these cases it is hardly possible to map the interaction just by matching their primary sequences [60]. However, much work is needed to determine the precise three-dimensional structures of thousands of large protein complexes. Promising steps in this direction are either based on combining results from pair wise docking of rigid proteins [68] or on combining experimental information with bioinformatics approaches [67]. On the other hand, the PRS discussed in this review are mostly short, extended peptides. For this case, both experimental methods (e.g., phage display or yeast two-hybrid) and computational approaches (e.g., pattern matching or flexible docking+refinement) can lead to reasonably accurate interaction data. For example, Cesareni and coworkers studied the binding of PRS with SH3 domains by a combined experimental and theoretical methods [69] and established an interaction network between different PRS and SH3 domains.

The weak but rapid binding of PRS to recognition domains makes the system an ideal object of developing protein function networks or protein function predictions [70,71]. In this review, we pointed out the importance of accounting for the multiplicity of interactions between PRS and the recognition domains and modifications of protein functions that depend on the isomerization of proline residues. Similar considerations may apply for peptide substrates of protein kinases as well. Here, we mention in particular the inhibitor of cAMP-dependent protein kinase, PKI, which is partly unstructured in the unbound form and folds upon binding to cAPK.

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