Biochimica et Biophysica Acta xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Multidomain Peptidyl Prolyl *cis*/*trans* Isomerases ☆

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ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form 10 November 2014
Accepted 13 November 2014
Available online xxxx

Keywords:
Peptidyl prolyl *cis*/*trans* isomerase
Multidomain
Cyclophilin
FKBP
Pin1

ABSTRACT

Background: Peptidyl prolyl *cis*/*trans* isomerases (PPIases) assist the folding and restructuring of client proteins by catalysis of the slow rotational motion of peptide bonds preceding a proline residue. Catalysis is performed by relatively small, distinct protein domains of 10 to 18 kDa for all PPIase families. PPIases are involved in a wide variety of physiological and pathophysiological processes like signal transduction, cell differentiation, apoptosis as well as viral, bacterial and parasitic infection.
Scope of review: There are multidomain PPIases consisting of one to up to four catalytic domains of the respective PPIase family supplemented by N- or C-terminal extensions. This review examines the biochemical and functional properties of the members of the PPIase class of enzymes which contain additional protein domains with defined biochemical functions.
Major conclusions: The versatile domain architecture of multidomain PPIases is important for the control of enzyme specificity and organelle-specific targeting, the establishment of molecular connections and hence the coordination of PPIase functions across the cellular network.
General significance: Accessory domains covalently linked to a PPIase domain supply an additional layer of control to the catalysis of prolyl isomerization in specific client proteins. Understanding these control mechanisms will provide new insights into the physiological mode of action of the multidomain PPIases and their ability to form therapeutic targets. This article is part of a Special Issue entitled Proline-directed Foldases: Cell Signaling Catalysts and Drug Targets.

1. Introduction

Many denatured, biologically inactive proteins can be refolded and reactivated *in vitro* in the absence of additional cellular components. However, the folding yield and the folding rate can differ significantly from the situation in the cell. It is now clear that a conformational interconversion, the *cis*/*trans* isomerization of peptide bonds (–C(=O)–NH– or –C(=O)–NR–), which is usually slow on the biological time scale, is often involved in the folding process [1]. Consequently, foldases, such as the prolyl peptide bond directed peptidyl prolyl *cis*/*trans* isomerases (PPIases) have evolved and were discovered in the 1980s [2]. A powerful biocatalytic rate acceleration of prolyl bond isomerization enables the folding dynamics of substrate proteins to be controlled by these enzymes. PPIases catalyze folding transitions of the polypeptide backbone in unfolded and partially folded polypeptide chains as well as in native state of proteins in response to an accessible proline residue in the amino acid sequence. Typically, rate accelerations of the enzymatic over the nonenzymatic isomerization were found up to 10^6 fold. The proposed foldase catalysis of the backbone conformation could mediate spatial and temporal control of bioactivity of a substrate protein.

Three ubiquitously distributed PPIase families, the cyclophilins, the FKBPs and the parvulins have been identified up to date [3]. These PPIase families have distinct substrate specificities and prove to be sensitive to different types of inhibitors. Cyclosporin A (CsA) and FK506, both naturally occurring small molecules, act as nanomolar, reversible inhibitors for most cyclophilins and FKBPs, respectively [4,5]. Early studies have led to the identification of cyclophilins and FKBPs implicated in the immunosuppressive properties of CsA and FK506 by gain of function involving the inhibition of the protein phosphatase 2B (calcineurin). This result gave rise to these families being called immunophilins [6,7]. Nonimmunosuppressive derivatives of these drugs which still strongly inhibit the PPIase activity of the respective enzymes have been developed. From numerous structural investigations it has become clear that the submolecular machinery required to catalyze a prolyl isomerization forms relatively small, distinct protein domains of 10 to 18 kDa for all PPIase families. This structural organization of biocatalysis might predispose other protein functions to recruit PPIase domains for foldase control of their bioactivity. Consequently, beside prototypic PPIases many multidomain isoforms have been identified in almost all organisms examined including bacteria, fungi, plants and animals. The multidomain

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http://dx.doi.org/10.1016/j.bbagen.2014.11.012
0304-4165/

PPIases comprise from one to up to four catalytic domains of the respective PPIase family supplemented by N- or C-terminal extensions of different functionalities. PPIases containing both, a cyclophilin and a FKBP domain have been only found in three protozoan parasites and in two bacteria so far, whereas parvulins and the other PPIase families do not come together to form a multidomain PPIase [8]. The versatile domain architecture of multidomain PPIases is probably important for the control of enzymatic specificity and organelle- and tissue-specific targeting, the establishment of molecular connections and hence the coordination of PPIase functions across cellular networks.

2. Functionality of domains recruited by multidomain PPIases

A protein domain is defined as a conserved part of a polypeptide chain that can exist, fold and function independently. Only eight known functional domains, the TPR domain, the WD40 domain, the U-box, the E3 SUMO ligase domain, the EF hand domain, the RRM domain, the WW domain and the Ran binding domain were found to be components of multidomain PPIases (Table 1). The U-box domain as a modified RING domain and the E3 SUMO ligase domain exhibit E3 ligase activity [9,10]. The RRM domain is a common RNA binding domain but can also interact with proteins. Ran binding domains are typical for the giant nucleoporin Cyp358 (RanBP2) and specifically bind the Ran GTP complex. The other four accessory domains in multidomain PPIases represent protein–protein interaction domains.

2.1. Multiple repeat protein scaffolds

The two multiple repeat protein scaffolds TPR (tetratricopeptide repeats) and WD40 (WD or beta-transducin repeat) have been found in combination with domains of two families of PPIases.

A TPR domain consists of multiple repeats of 34 amino acids sharing a degenerate consensus sequence defined by a specific pattern of hydrophobic amino acid side chains. Adjacent TPR motifs assemble into parallel helix–turn–helix arrangements [11]. Cluster of three consecutive TPR motifs are the most common organization of the repeat. Multiple TPR domains generate a right-handed superhelix. This creates a groove with a large surface area available for ligand binding [12]. TPR motifs are the only modules described to be associated with two PPIase families, the cyclophilins and FKBPs. Six human FKBPs and two human cyclophilins contain TPR motifs. Interestingly, in the PPIases in protozoan parasites, which contain a cyclophilin and a FKBP domain, both domains are interconnected via a TPR domain [8]. *Saccharomyces cerevisiae* in contrast to mammals has not evolved TPR domain FKBPs but does contain TPR domain cyclophilins.

The TPR domains have been found to mediate the interaction of multidomain PPIases with Hsp90, thus facilitating the organization of the respective PPIases in Hsp90 heterocomplexes [13]. The TPR domains of FKBP38, FKBP52, Cyp40, and other TPR domain proteins directly bind to the Ca^{2+}/S100 proteins in a Ca^{2+}-dependent manner. This competitive interaction leads to the dissociation of the PPIases from Hsp90 [14]. In the Hsp90 heterocomplexes, the PPIase domains are considered to target the respective Hsp90 client proteins. Therefore, most likely the PPIase anchored by the TPR domain in a multiprotein complex can catalyze a conformational transition required for the function of the complex. Contradictory to this hypothesis, the active site mutations Y57A, F67Y, W90L, and F130Y of FKBP52 had no effect on FKBP52-dependent potentiation of glucocorticoid receptor and androgen receptor [15]. It is important to note, that substitutions of the respective active site residues in FKBP12 reduce the activity only 10 to 20fold in a tetrapeptide based PPIase assay [3]. Thus, it could be possible that the residual PPIase activity of the FKBP52 variants is sufficient to elicit the receptor response, especially if encounter complex formation is supported by a second binding site on the proteinaceous substrate. The FD67DV double mutation in the FKBP52 active site strongly reduced the influence of FKBP52 on both receptors; however its impact on PPIase activity is not described [15].

WD40 repeats are repetitive sequence motifs usually about 40 amino acids in length forming four-stranded antiparallel β-sheets. In a protein chain WD40 repeats fold into a β-propeller architecture, which can principally contain four to eight WD40 repeats oriented around a central axis. The WD40 domain was shown to interact with large globular proteins and with shorter peptide segments in a way involving the participation of its entire surface. This property might enable the PPIase domain to control scaffolding and the assembly and regulation of protein complexes [16].

In Cyp73 the C-terminal cyclophilin domain is supplemented by a WD40 domain with four WD40 repeats. Orthologs of Cyp73 can be found in a wide variety of eukaryotes but not in *S. cerevisiea* whereas WD40 domains generally are abundant in eukaryotic proteomes.

2.2. EF hands

The EF-hand motif is the most common Ca^{2+}-binding motif found in proteins [17]. The sequence of the EF-hand motif comprises approximately 40 residues forming a helix–loop–helix structural unit: two a-helices connected by a Ca^{2+}-chelation loop. The EF-hand motif often occurs in pairs that together form a four-helix bundle domain. In several EF hand proteins, Ca^{2+}-binding induces structure formation. More specifically in most EF-hand proteins Ca^{2+}-binding leads to a conformational change through, which this protein transmits the message of an increased Ca^{2+} concentration. In these Ca^{2+} sensor proteins, conformational changes upon Ca^{2+}-binding usually trigger the interaction with a target protein [18].

Amongst human PPIases, only FKBPs are found supplemented by EF hand domains. All four human EF hand FKBPs localize to the ER lumen, which forms an intracellular Ca^{2+} store. The decrease of the Ca^{2+} concentration in the ER was shown to induce degradation of FKBP65 suggesting Ca^{2+}-binding by the EF hand domain to be important for enzyme stability [19]. Apparently, *Arabidopsis thaliana* and *S. cerevisiae* completely lack EF hand domain PPIases.

2.3. RRM domains

The RNA recognition motif (RRM) is an abundant protein domain in eukaryotes, which consists of about 90 amino acids adopting a αβ sandwich structure [20]. It contains two characteristic consensus sequences, RNP1 and RNP2, in the center and at the N-terminus of the domain. RRM domains are not only involved in sequence- and shape-dependent RNA recognition but also in protein–protein interactions [20]. There are two human RRM containing cyclophilins. The RRM domain of Cyp33 has been found to associate independently with both, poly(A) and poly(U) RNA sequences and the histone methyltransferase MLL1. The

Table 1
The occurrence of functional domains in different human PPIases.

Functional domain	Human PPIase
TPR domain	Cyp40 (PPID)
	FKBP36 (FKBP6)
	FKBP37 (AIP)
	FKBP38 (FKBP8)
	FKBP44 (AIPL1)
	FKBP51 (FKBP5)
	FKBP52 (FKBP4)
WD40 domain	Cyp73 (PPWD1)
U-box domain	Cyp60 (PPIL2, Cyc4)
E3 SUMO ligase domain	Cyp358 (RanBP2)
RRM domain	Cyp33 (PPIE, CypE) Cyp57 (PPIL4)
Ran binding domain	Cyp358 (RanBP2)
EF hand domain	FKBP22 (FKBP14)
	FKBP23 (FKBP7)
	FKBP63 (FKBP9)
	FKBP65 (FKBP10)
WW domain	Pin1

Please cite this article as: C. Schiene-Fischer, Multidomain Peptidyl Prolyl *cis/trans* Isomerases, Biochim. Biophys. Acta (2014), http://dx.doi.org/10.1016/j.bbagen.2014.11.012