Left-handed polyproline-II helix revisited: proteins causing proteopathies

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Left-handed polyproline-II type helix is a regular conformation of polypeptide chain not only of fibrous, but also of folded and natively unfolded proteins and peptides. It is the only class of regular secondary structure substantially represented in non-fibrous proteins and peptides on a par with right-handed alpha-helix and beta-structure. In this study, we have shown that polyproline-II helix is abundant in several peptides and proteins involved in proteopathies, the amyloid-beta peptides, protein tau and prion protein. Polyproline-II helices form two interaction sites in the amyloid-beta peptides, which are pivotal for pathogenesis of Alzheimer’s disease (AD). It also with high probability is the structure of the majority of tau phosphorylation sites, important for tau hyperphosphorylation and formation of neurofibrillary tangles, a hallmark of AD. Polyproline-II helices form large parts of the structure of the folded domain of prion protein. They can undergo conversion to beta-structure as a result of relatively small change of one torsional angle of polypeptide chain. We hypothesize that in prions and amyloids, in general polyproline-II helices can serve as structural elements of the normal structure as well as dormant nuclei of structure conversion, and thus play important role in structure changes leading to the formation of fibrils.

Keywords: PPII; polyproline-II helix; proteopathies; prion protein; beta-amyloid; protein tau

1. Introduction

Left-handed polyproline-II type (PPII) helix has emerged as a result of over 25 years of protein structure research as one of the principal classes of regular secondary structure (local structure) of polypeptide chain in peptides (Drake, Siligardi, & Gibbons, 1988; Esipova, Lobachev, Rogulenko, & Shibnev, 1984; Makarov, Esipova, Lobachov, Grishkovsky, & Pankov, 1984; Rath, Davidson, & Deber, 2005; Woody, 1992), and proteins (Adzhubei & Sternberg, 1993; Adzhubei, Sternberg, & Makarov, 2013; Hollingsworth, Berkozl, & Karplus, 2009). The designation PPII-helix is in part misleading, for though there is high preference for Pro residues to be found in stretches of PPII helices, the presence of proline is not a prerequisite for PPII (Hollingsworth, Lewis, Berkozl, & Karplus, 2012). Indeed, it was shown that up to 46% of PPII helices in folded proteins do not contain proline (Cubelli, Caillez, Blundell, & Lovell, 2005). In folded proteins though the PPII helix is substantially less frequent compared to alpha-helix and beta-strand, it is the only class of regular structures occurring on a comparable scale (Adzhubei et al., 1987; Adzhubei & Sternberg, 1993; Jha et al., 2005). In the natively unfolded proteins and unstructured peptides, PPII is one of the most populated conformations maintaining the local order (Adzhubei et al., 2013; Schweitzer-Stenner, 2012; Shi, Chen, Liu, & Kallenbach, 2006). PPII-helix is the most extended helical structure in proteins (twice the length of alpha-helix per residue) and flexible since it does not form regular networks of hydrogen bonds. PPII helices underlie a range of functions of proteins; apart from providing flexible structural building blocks, perhaps the most important of these functions is mediating protein–protein and protein–nucleic acid interactions.

Proteopathies can be described as protein conformational disorders triggered by conformational changes resulting in aggregation, with various forms of aggregates affecting tissues and organs of the nervous system, subsequently disrupting vital functions of the organism.

2. Methods

2.1. PPII-helix assignment (annotation)

PPII helices have been annotated for three-dimensional structures available as PDB formatted files, using the PolyprOnline server (Chebrek, Leonard, de Brevern, & Gelly, 2014). For the prion structure PDB 4F37, the presence of PPII helices in Abeta(1-16) was reported by the authors (Nisbet et al., 2013). PPII annotation for Abeta(1-16) as part of this structure has been performed with PolyprOnline server supplemented with the PPII assignment according to Adzhubei and Sternberg (1993).

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Type of protein secondary structure is calculated by PolyprOnline server using coordinates of the atoms in structures presented as PDB formatted data. For Abeta, these structures generally contained not the whole Abeta (1-42) peptide, but its fragments. Fragments in different structures were different, so the contribution to the statistics from different parts of the Abeta peptide sequence depended on the coverage of this area by the structures. The resulting data were normalized to address this issue.

2.2. Normalization

Occurrence of each amino acid residue in sequence (e.g. sequence of Abeta(1-42)), in all structures used in annotation was calculated (structure coverage is given in Tables 1 and 2). PPII annotation was performed by PolyprOnline server using three methods: DSSP, Pross and Segno, producing three different annotations for each structure. Normalization was performed by dividing the total number of PPII assignments for each residue in the sequence by the number of structures where this residue appears (defined by structure coverage), and by the number of annotations for each structure (three annotations). The resulting value range is from 0 to 1. In the case when the residue was present in at least one structure, and all the methods assigned this residue in all structures as a PPII conformation, the normalized occurrence value was 1. If the residue was found at least in one structure, but had no PPII conformation assignments, the normalized occurrence value was 0. In the case when the residue did not appear in any structure, normalized occurrence value was also set to 0.

Table 1. Abeta sequence coverage by experimentally solved structures.

| Sequence  | D | E | F | R | H | D | S | G | Y | E | V | H | H | Q | K | L | V | F | F | A |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X-ray     | 8 | 15| 13| 13| 12| 9 | 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 10| 9 | 10| 10| 10| 9 |
| NMR nonpolar | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 2 | 2 |
| NMR micelle | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| NMR DMSO  | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| NMR water | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Solid-state NMR | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 5 | 5 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 |
| Total     | 22| 29| 27| 27| 26| 23| 20| 19| 20| 21| 21| 23| 23| 25| 34| 34| 34| 34| 34| 34| 34|

| Sequence  | D | E | F | R | H | D | S | G | Y | E | V | H | H | Q | K | L | V | F | F | A |
|-----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| X-ray     | 4 | 3 | 1 | 1 | 1 | 2 | 2 | 4 | 4 | 4 | 4 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 2 |
| NMR nonpolar | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| NMR micelle | 8 | 8 | 8 | 9 | 9 | 9 | 5 | 5 | 5 | 5 | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 |
| NMR DMSO  | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| NMR water | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | 2 | 2 | 2 | 2 | 0 | 0 |
| Solid-state NMR | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| Total     | 28| 27| 25| 26| 26| 26| 27| 23| 25| 25| 25| 25| 25| 25| 22| 22| 22| 22| 22| 22| 8 |

Notes: Numbers represent the number of structures that include an amino acid residue.

NMR nonpolar: solution NMR in nonpolar solution; NMR micelle: solution NMR in water-micelle and membrane-like environment; NMR DMSO: solution NMR in bipolar solution of 100% DMSO-d6. Details of the data-set are given in Supplementary Table 1S.

2.3. Datasets

Details of the three-dimensional structures used in this study are given in Supplementary Table 1S.

2.4. Structure modelling

Structure modelling was performed using servers Hhpred (Söding, Biegert, & Lupas, 2005), iTasser (Roy, Kucukural, & Zhang, 2010), ModWeb (Pieper et al., 2014), Phyre2 (Kelley & Sternberg, 2009) and RaptorX (Källberg et al., 2012), with subsequent quality assessment of the obtained models and building of the final model by the QA-RecombineIt server (Pawlowski, Gajda, Matlak, & Bujnicki, 2008). The structure of (α7)5 was the Swiss-Model (Biasini et al., 2014) based on the PDB 5AFN (sequence identity 64%).

2.5. Prediction of interactions by docking

Structure of Abeta(1-16) peptide solved as part of the PDB 4F37 crystallographic structure (2.57 Å) was used. For target proteins, experimentally solved structures were used where available, otherwise server and expert models were built (Supplementary Table 1S). Global docking was performed with Abeta(1-16) as ligand and target proteins as receptors. Each docking job Abeta(1-16)–target protein has been submitted to servers Cluspro (Kozakov et al., 2013), HEX (Ghoorah, Devignes, Small-Tabbone, & Ritchie, 2013), SwarmDock (Torchala, Moal, Chaleil, Fernandez-Recio, & Bates, 2013) and Zdock (Pierce et al., 2014), each run producing 10 best models. The models obtained from all servers formed the predictions of dataset, where ligand–receptor atomic
interactions were calculated for each complex model. To assess average number of atomic interactions for each residue position in Abeta(1-16) sequence in the set of docking models, the overall number of Abeta(1-16)—target protein atomic interactions for all models, for each residue involved in intermolecular interaction was calculated. The number of atomic interactions for each amino acid residue in Abeta(1-16) is the total number of interactions for this residue in docking models where interaction for this residue was identified. Clusters of residues with high numbers of interactions indicated putative interaction sites.

3. Prion diseases
In prion diseases, dramatic rearrangements take place in the structure of glycosylated cellular prion proteins (PrPC), a physiologically normal form of the prion protein (PrP) localized at the cell surface (Bella, Hindle, McEwan, Lovell, 2008; Prusiner, 1998). These changes lead to PrPC conversion into insoluble and protease-resistant pathogenic isoform (PrPSc). PrPSc aggregation results in the formation of oligomers and subsequently amyloid fibrils, and causes spongiform encephalopathies e.g. such human diseases as Creutzfeldt–Jakob disease, Kuru and Gerstmann–Sträussler–Scheinker syndrome, as well as spongiform encephalopathies of animals. In accordance with the protein hypothesis, PrPSc proteins are capable of inducing transition of PrPC into the pathogenic form, i.e. they act as the infectious agent of the disease.

PrPC has the partially natively unfolded N-terminal domain; the rest of the protein is folded and predominantly alpha-helical. Spectroscopic studies of the N-terminal domain show local structure of the polypeptide chain formed by PPII-helix and beta-turns (Blanch et al., 2004; Gill et al., 2000). The segment of PrPSc that can act as transmissible agent includes amino acid residues 90–231(human prion sequence numbering) (Prusiner, 1998). Structure rearrangement in PrPC according to numerous models leads to the formation of beta-structure-based fold that incorporates the N-terminal domain, but as yet there is no full experimentally solved structure (Requena & Wille, 2014). There is a range of models built using data of biophysical methods including solid-state nuclear magnetic resonance spectroscopy (NMR). The PrPSc structure in these models is represented either by beta-sheets and alpha-helices or solely by beta-sheets (Diaz-Espinoza & Soto, 2012; Huang, Prusiner, & Cohen, 1996; Requena & Wille, 2014).

3.1. Results and discussion
We hypothesized earlier that PPII can play an important role in the physiologically normal structure of PrPC and in its conversion to the PrPSc (Adzhubei et al., 2013). To ascertain occurrence and localization of PPII-helix, we have annotated PPII in the experimentally solved crystallographic (X-ray) and NMR prion structures available from Protein Data Bank (PDB) (Berman, Henrick, Nakamura, & Markley, 2007). Annotation has been performed with the PolyprOnline server (Chebek et al., 2014).

PPII occurrence data obtained from the 11 human prion (Human major prion protein) PrPC X-ray structures are shown in Figure 1(a), and localization of the PPII helices in the prion PDB structure 4KML is shown in Figure 2. Details of the data-set are given in Table 1S. PPII stretches in human prions have been identified in all interconnecting loops, positioned between beta-strands and alpha-helices in prion structure (Figures 1(a) and 2). Long, curved PPII helix forms most of the N-terminal part of prion resolved in the X-ray structures. Thus, PPII helices in prions serve as major structural building blocks. Residues in PPII conformation are also present in substantial numbers in the beta-strands forming beta-sheets (Figures 1(a) and 2). Although such occurrences had been reported previously (Jha et al., 2005), in prions the number of PPII in beta-strands is unusually high.

Structure annotation of prions shows that they are highly saturated with PPII. Percentage of the PPII content as annotated e.g. in the PDB 4KML structure is 8.6%, compared to the 2–4% average depending on the length of PPII helices (3 and 4 and more residues) and reported in structure surveys (Adzhubei & Sternberg, 1993; Stapley & Creamer, 1999).

In addition to human prion structures, PPII annotation has been also carried out for 13 other species. Annotated sequence alignments for 24 crystallographic and NMR structures of PrPC are shown in Figure 1(b). The results show that the PPII regions overall are conserved across different species. These results support the evolutionary
conserved role of PPII structure in PrPC; they are in accord with PPII conservation data obtained for other protein families (Adzhubei & Sternberg, 1994). Notably, relatively better PPII conservation is observed in crystallographic structures compared to NMR. This might be a result of different environment conditions in crystals and solution samples, and reflect different structural states. However, these discrepancies can also reflect differences

Figure 1. PPII annotation for prion structures. (a) PPII assignment results for the experimentally solved structures of human prion protein, annotation for 10 crystallographic structures. Bars show normalized annotated PPII occurrence for each amino acid. (b) PPII assignment for prion structures of different species. PPII-helices are underlined. Note: Sequence alignment shows conservation of the PPII-helices. Structures used for annotation: x – crystallographic structures only, xn – crystallographic and NMR, n – NMR structures only.
in parameters used in the NMR structure computations, where certain parameter sets might not incorporate PPII as a distinct structure class.

The discovered abundance and conservation of PPII in prions corroborate our suggestion that it plays an important role in prions. PPII is a flexible structure and its geometry and lack of rigid network of stabilizing hydrogen bonds make it possible for this structure to undergo swift conformational transitions (Adzhubei et al., 2013).

According to Kozin et al. (2001) and Prusiner (2001), the region of prion structure that includes alpha-helix-H1 followed by the loop and beta-strand-B2, (which are in part formed by PPII) is crucial for prion structure rearrangement leading to the emergence of PrPSc. Circular dichroism and NMR spectroscopy studies of the synthetic peptide corresponding to this fragment in sheep prion protein were conducted (Bertho, Kozin, Debey, Hoa, & Girault, 2001; Kozin et al., 2001) and it was observed that in solution the peptide adopted beta-hairpin structure formed by anti-parallel beta-strands; sequence-wise the N-terminal strand in the peptide matched alpha-helix-H1 in PrPC structure. They also suggested that alpha-helix-H1 and the preceding loop formed a highly stable structural motif in PrPC (Lepage et al., 2004) (PDB 1S4T), and once this motif is disrupted alpha-helix-H1 can unfold and the part of PrPC structure that starts from the N-terminal beta-strand and extends to the beta-strand preceding alpha-helix-H2, forms anti-parallel beta-sheet, resulting in the PrPSc structure (Kozin et al., 2001). We have found in this study that the beta-strands and loops in PrPC have high content of PPII, and that PPII-helix precedes alpha-helix-H1, forming a PPII-helix – alpha-helix structural motif also found in other proteins, e.g. in the high-resolution crystallographic structure of avian pancreatic polypeptide (Glover et al., 1983).

It seems feasible to hypothesize that in prions, PPII helices are part of the mechanism responsible for equilibrium between the PrPC and PrPSc structures. The presence of PPII helices might be linked with the physiologically normal structure of PrPC where they form flexible building blocks; they at the same time represent the only conformation of polypeptide chain potentially capable of fast transition to beta-strands. PPII with the exception of Pro residues (there are only three conserved Pro in PPII in prions) can re-fold from the left-handed PPII-helix to right-handed beta-strand by a relatively small change in a single torsional angle, phi. In PrPC structure, PPII helices I and II already overlap with the short beta-strands forming beta-sheet (Figure 2), and following such conformational transition, beta-strands can extend to the PPII regions III, IV and V, forming beta-sheet characteristic of PrPSc. This is in accord with the models introduced in (Bertho et al., 2001; Huang et al., 1996; Prusiner, 2001; Wille et al., 2002). Conversion of the N-terminus PPII helices to beta-structure is also consistent with the models described in (Diaz-Espinoza & Soto, 2012; Requena & Wille, 2014).

4. Alzheimer’s disease

Currently widely accepted amyloid hypothesis implies that morphologically the main pathogenic agents causing Alzheimer’s disease (AD) are amyloid plaques located in the brain and mainly formed by beta-amyloid aggregates (Duyckaerts, Delatour, & Potier, 2009; Holtzman, Morris, & Goate, 2011; Kulikova, Makarov, & Kozin, 2015). Beta-amyloid (Amyloid beta or Abeta) peptides form dimers and oligomers, which subsequently aggregate. Common species of beta-amyloid peptides are the Abeta (1-40) and Abeta(1-42), but also Abeta(1-16). These extracellular peptides are produced as a result of processing of the amyloid precursor protein. The peptides are
also present in blood and cerebrospinal fluid under physiologically normal conditions. The other pathological manifestations that can serve as a marker of AD are neurofibrillary tangles (NFTs) composed of paired helical filaments (PHFs), formed by aggregates of hyperphosphorylated protein tau in the brain cells (Duyckaerts et al., 2009; Holtzman et al., 2011).

Tau proteins are mostly present in CNS neurons and their physiological function is to stabilize microtubules in axons. Abnormal tau proteins are involved in a number of neurodegenerative disorders – tauopathies (Kovacs, 2015; Murray et al., 2014), where pathologic condition is associated with NFTs (PHFs). NFTs are formed as a result of hyperphosphorylation of tau leading to aggregation of the misfolded protein; it should be noted that there is no universally accepted notion that formation of NFTs is directly harmful for the brain. AD is perhaps one of the most important tauopathies, but it is also not yet clear whether formation of amyloid plaques or NFTs plays primary role in AD. Tau is a natively unfolded protein (Wang & Mandelkow, 2015), and there is Raman and Raman optical activity spectroscopic evidence suggesting that PPII is a major conformation of its local structure, with significantly lower content of beta-strands (Syme et al., 2002). This is consistent with the fact that tau sequence is enriched in proline, abundant in its proline-rich domains (Kolarova, García-Sierra, Bartos, Ríncy, & Ripova, 2012). Some proline residues are also located in the C-terminal region of pseudo-repeats. Tau phosphorylation sites are largely located in the proline-rich and C-terminal domains (Kolarova et al., 2012), majority of these sites are formed by the Ser-Pro and Thr-Pro motifs (Buée, Bussière, Buée-Scherrer, Delacourte, & Hof, 2000). Proline-rich regions display high propensity for PPII, which is even higher for the sites expressly incorporating Pro. PPII-helix thus emerges as highly preferable local structure of the Tau protein phosphorylation sites.

4.1. Results and discussion

4.1.1. Abeta peptides

Solving experimental structures of the peptide Abeta is difficult, since under physiological conditions in solution, the Abeta peptides are prone to polymerization and aggregation. There is however a large number of Abeta structures of different lengths available from PDB, mostly short peptide fragments. These structures were solved under various environment conditions, more or less different from the physiological conditions, and as complexes with other proteins. We assessed the following structures available from PDB (September 2015): 27 crystallographic structures, 19 solution NMR and 7 solid-state NMR structures. There are also five model structures which have not been included in this survey. Structure coverage for Abeta is presented in Table 1.

Of the Abeta NMR structures, 3 structures were solved in nonpolar solvent, 9 in membrane-like and micelle environment, 4 in water solution and 3 in a biphasic solvent dimethyl sulfoxide (DMSO). In the two of the crystallographic structures, peptides Abeta(1-16) and Abeta(18-41) form part of chimeric proteins. Details of the data-set of structures are given in Supplementary Table S1.

The aim of the survey was to identify PPII helices in the structures of Abeta solved under wide range of conditions. Such analysis also provides insight into how stable the PPII-helix is as a structural element of this relatively short peptide fragments in the samples with various physical and chemical characteristics that were used for the experimentally solved structures. PPII occurrence annotation has been performed with the PolyprOnline server for the data-set of 53 X-ray and NMR structures; the results are shown in Figure 3(a).

Major cluster of PPII occurrence in the annotation data is 2-AEFRHDS-8 (I) close to the Abeta N-terminus. The PPII-helix in this cluster includes residue H6 chelating zinc and copper ions in the Abeta(1-16) peptide (Tõugu, Tiiman, & Palumaa, 2011). The other cluster within the Abeta(1-16), 10-YEVHHQ-15 (II) has markedly lower PPII occurrence in the annotated structures compared to cluster I. This cluster includes zinc and copper binding sites (UNIPROT database assignment for P05067 (A4_HUMAN)), in particular the zinc-binding structural motif 11-EVHH-14 important for Abeta dimerization (Tsvetkov et al., 2010). PPII helices corresponding to clusters I and II are shown in Abeta(1-16) from the PDB 4F37 X-ray structure in Figure 4. The other PPII clusters are 19-FFFAE-22 (III), 24-VGVSNGAI-31 (IV). Two O-glycosylation sites, S8 and S26 (UNIPROT assignment) lie in the clusters I and II which is in accord with our earlier conjecture that PPII structure can be favourable for O-glycosylation sites (Adzhubei et al., 2013).

PPII annotation results for Abeta crystallographic and NMR structures solved under different conditions are shown in Figure 3(b), (c) and (d). In the crystallographic structures data (Figure 3(b)) the PPII clusters are more distinct, with the highest PPII occurrence still in the N-terminal cluster I, followed by much more narrow clusters II and IV. PPII occurrence in cluster III is low. Cluster I dominates the solid-state NMR annotations, with significantly less PPII annotated in cluster II and clusters III and IV are even less populated and represented by single residues (Figure 3(d)). Solution NMR data show a more fuzzy picture (Figure 3(e)), perhaps because structure coverage for each set of environment conditions was low. Most of the PPII is annotated in cluster I in all media, with high occurrence for water, micelles and DMSO. PPII has the highest annotation rate for the Abeta NMR DMSO structures, with contributions
in clusters I, II, III and IV. Cluster II is less pronounced and includes contributions from the DMSO, micelles and nonpolar solvent structures. Cluster III is annotated only for the NMR DMSO structures. Cluster IV is formed by contributions from the DMSO, micelles, water and nonpolar solvent structures.

For the solution NMR, PPII has the highest annotation rate for the Abeta DMSO structures, with contributions in clusters I, II, III and IV. From the PPII clusters I and II covering functionally important regions of Abeta(1-16), cluster I is consistently represented in the structures solved by crystallography, solid-state NMR and solution NMR with various sample conditions. Functionally important stretch of cluster II, 11-EVHH-14 is annotated as PPII in the X-ray and solid-state NMR. In the solution NMR DMSO, micelles and nonpolar structures, residues in PPII conformation are present only in the fringes of this fragment.

Overall, PPII assignment shows surprisingly consistent presence of PPII in practically all available Abeta PDB structures across the range of experimental techniques and sample parameters. Thus, analysis of the experimentally solved structures showed that PPII forms several segments of Abeta local structure, and that the PPII-helix located at the N-terminus has the highest annotation rate. PPII helices form functionally important sites, e.g. sites implicated in metal binding.

There are two structures of the beta-amyloid fibrils available, the solution NMR, PDB 2BEG (Abeta(17-42)) and solid-state NMR, PDB 2M4J (Abeta(1-40)). Analysis of these structures show that all clusters, I, II, III and IV partially retain PPII conformation, which does not disrupt structure of the fibrils. Thus, hypothetically PPII helices in amyloid fibrils can either undergo structural conversion to beta-strands, or retain their conformation and form flexible blocks within beta-sheets.
PPII helices are known as a structure favourable for binding sites in proteins (Siligardi & Drake, 1995). Experimental evidence exists indicating that beta-amyloid interacts with a range of proteins including serum albumin, prion protein, beta-2-microglobulin, extracellular domains of neuronal nicotinic acetylcholine receptors (α7)5 and (α4)2(β2)3, prołow-density lipoprotein receptor-related protein 1 clusters II and IV, advanced glycosylation product-specific receptor (RAGE) extracellular domain and N-formyl peptide receptor 2 (FPRL2 or FPRL1) extracellular domain. To study the role of PPII-helices present in Abeta as potential binding sites in these interactions, we have modelled Abeta(1-16) binding with these proteins. Modelling has been performed by docking, using as a ligand Abeta(1-16) from the 2.57 Å resolution crystallographic structure PDB 4F37, which is the only available crystallographic structure covering the full length of Abeta(1-16) (Figure 4). Experimentally solved structures of these proteins have been used, and if they were not available, structures have been modelled. Full list of these proteins and details of their three-dimensional structure are given in Table 1S. Interaction of Abeta(1-16) with each of these proteins has been modelled by global docking using four different docking servers, Cluspro, HEX, SwarmDock and Zdock. Full set of the best ranking – target protein complex, produced – final prediction was based on a combined data of these models, it was more reliable than prediction obtained using a single server. Figure 5 shows number of interactions per residue for Abeta(1-16) modelled by docking to receptor proteins. Highest numbers of interactions, denoting the preferable binding site, have been predicted for residues in the PPII-helix 3-EFRH-6 in all models of complexes, except binding with neuronal nicotinic acetylcholine receptor (a)4(β2)3 (Figure 5(a)), where comparable numbers of interactions have been shown for residues 14-HQK-16 at the C-terminus, and prion protein (Figure 5(i)). Residue Y10 displays equally high number of interactions in the majority of models except neuronal nicotinic acetylcholine receptors and serum albumin (Figure 5(a), (b), and (g)). The preference of PPII-helix 3-EFRH-6 as a predicted location for binding site in Abeta(1-16) is consistent with the peptide structure, where it is more exposed and flexible in comparison with the more rigid (Nisbet et al., 2013) PPII-helix 11-EVHH-14, which forms part of a loop, is shown in Figure 4.

In the model of Abeta(1-16)–prion complex, although there is substantial involvement of Abeta(1-16) PPII-helix 3-EFRH-6, the highest number of interactions is centred on Y10. In the target prion protein (PDB 4KMLA), three areas of interactions have been predicted (data not shown). The more pronounced cluster with the highest number of predicted interactions is positioned at the interaction interface formed by the following structure blocks: beta-strand/PPII-I and PPII-IIIa; C-terminus of alpha-helix-H1 and the following loop including PPII-IV; C-terminus of alpha-helix-H2 (Figure 2). There are also less populated but distinct interaction clusters, which cover the groove formed by PPII-IIIb and alpha-helix-H1, and the opposite face of alpha-helix-H1 from PPII-IIIb (Figure 2). These interactions involve PPII helices that are characteristic of the prion N-terminus. The other smaller interaction cluster is centred on the 3–10 helix following beta-strand/PPII-IV and the C-terminus of alpha-helix-H3 (Figure 2). The presence of several highly populated clusters indicates that in prion there are possible multiple interaction with Abeta(1-16), and location of these clusters point to possibility of interactions PPII-helix – PPII-helix.

4.1.2. Protein tau

Since tau protein is natively unfolded, there are experimentally solved structures only of short segments available in PDB. We have performed annotation of PPII occurrence in four such X-ray and NMR structures, jointly covering segment 528-RTPSLPTPPTREPKK-VAVVRTPPKPS-552, located in the proline-rich domain P2 (UNIPROT P10636 (TAU_HUMAN), isoform P10636-1), Figure 6, Table 2. Phosphoserine and phosphothreonine residues present in this segment at positions 529, 531, 534, 548 and 552 (UNIPROT annotation) are located in continuous PPII-helices, which form local structure of virtually the whole segment. These results support the inference that stretches of PPII-helix form structure of the tau protein phosphorylation sites.

There is experimental evidence that NFTs are formed via transition of some of the tau structure to beta-sheet (Barghorn, Davies, & Mandelkow, 2004; von Bergen, Barghorn, Biernat, Mandelkow, & Mandelkow, 2005), and aggregation of tau into PHFs. Although in principle, PPII is perfectly suited to serve as a precursor structure for such transition it can only proceed if PPII helices do not include proline residues. Hyperphosphorylation of tau takes place before its cleavage (Mondragón-Rodríguez et al., 2008; Saito et al., 2010) and increases its PPII propensity (Chin, Toptygin, Elam, Schrank, & Hilser, 2016). Tau cleavage precedes NFT formation (Rohn et al., 2002). In the course of the disease and formation of NFTs, there are two truncations of the C-terminus, at Asp421 (UNIPROT P10636-8) and Glu391 (UNIPROT P10636-8). These truncation events leave intact the bulk of the protein, including its
Proline-rich domains and pseudorepeats. Hexapeptides PHF6 (306-VQIVYK-311 (UNIPROT P10636-8)) and PHF6* (275-VQIINK-280 (UNIPROT P10636-8)) at the beginning of R2 and R3 repeats have high propensity to undergo conversion to beta-structure, and represent the minimal tau aggregation sites; they can act as binding motifs in the formation of PHFs (von Bergen et al., 2000, 2005). X-ray and spectroscopic studies show limited presence of the beta-structure (Bartholomew et al., 2004; Kirschner, Abraham, & Selkoe, 1986; Schweers, Schönbrunn-Hanebeck, Marx, & Mandelkow, 1994), indicating that the majority of structure of tau in the PDFs is not involved in beta-sheets (von Bergen et al., 2005). There is also solid-state NMR data suggesting that the fibril core is built mainly by the repeat R3 (Andronesi et al., 2008).

Figure 5. Putative interaction sites in Abeta(1-16) predicted by docking. Bars represent number of atomic interactions in Abeta predicted for each amino acid. Note: PPII helices are marked dark grey with their N- and C-terminal residues marked with oblique hatching. (a) Neuronal nicotinic acetylcholine receptor (α4)2(β2)3 extracellular domain (4nAChR). (b) Neuronal nicotinic acetylcholine receptor (α7)5 extracellular domain (7nAChR), (c) LRP cluster II, (d) LRP cluster IV. (e) RAGE ectodomain. (f) FPRL1 (FPR2) extracellular domain. (g) Serum albumin. (h) Beta-2-microglobulin. (i) Prion protein.
Taken together these results indicate that PPII helices have a different role in NFTs in comparison with their proposed involvement in prion structure rearrangement and formation of amyloid fibrils by beta-amyloid peptides. Since PPII helices in tau are mostly proline-rich, there is no transition of PPII to beta-strands, which hinders formation of intermolecular beta-sheets in the parts of structure where they are located. PPII helices in tau serve as structural blocks ensuring that a larger part of the protein in NFTs remains unfolded.

5. Conclusions

The focus of this study has been on several proteins and peptides that either cause or have crucial impact on the pathogenesis of a number of proteopathies. It has been demonstrated that PPII-helix is abundant in all proteins that have been surveyed in this study. It represents a major structural class in the three-dimensional structures that have been analysed.

Function of PPII-helix stems from its properties as a highly extended structure, flexible since it is not entangled in regular networks of intra- or inter-chain hydrogen bonds, and in most instances exposed and hydrated (Adzhubei & Sternberg, 1993; Berisio, Loguercio, De Simone, Zagari, & Vitagliano, 2006). One of the most important PPII functions is serving as the structural motif for interactions in peptides and proteins. We have shown that PPII helices form two binding sites in the peptide Abeta(1-16) which are responsible for the metal-binding ability and dimerization of the peptide (Istrate et al., 2016), and hence are crucial for the pathogenesis of AD. We have further found that these PPII-helices are present in the other species of Abeta, i.e. Abeta(1-40) and Abeta(1-42). Interaction predictions by docking have demonstrated that the N-terminal PPII-helix in Abeta(1-16) is a preferred binding site in complexes of Abeta with a range of proteins and receptors that were reported to be involved in interactions with beta-amyloid.

PPII helices play important functional role in the other protein central to AD – tau protein, where they are abundant in proline-rich regions. Hyperphosphorylation of tau leads to aggregation of the misfolded protein forming NFTs, and PPII with high probability is the structure of the majority of tau phosphorylation sites. Proline-rich PPII helices cannot undergo conversion to beta-strands, thus in NFTs PPII helices form the unfolded part of their structure.

We have shown in this study that the folded domain of prion protein is saturated with PPII helices, especially in the N-terminal region. PPII-helices transverse long tracts of prion structure, they participate in beta-structure and form beta-type hydrogen bonds. PPII helices are to a large extent conserved in PrPC of a range of species. PPII-helix because of its characteristics and geometry is a mobile structure, well suited to undergo conformational transitions to beta-strand (Adzhubei & Sternberg, 1993; Adzhubei et al., 2013).

Their function in prions can be to serve as structural elements of the normal structure as well as the dormant nuclei of structure conversion to the prion misfolded state, that ensure possibility of vast structure changes leading to the formation of fibrils. In prions, they are in such reciprocal positions relative to each other and the beta-strands and alpha-helix-H1, that as a result of conformational transition PPII to beta-strand they can serve as elements of the prion conversion to the structure dominated by beta-sheets.

There is also possible involvement of PPII in impeding the formation of amyloid. e.g. it was suggested that high Pro–Gly content in sequences of elastin, amyloids, spider silks, wheat gluten, and insect resilin prevents formation of amyloid (Rauscher, Baud, Miao, Keeley, & Pomès, 2006). Such sequence bias could signify the presence of proline-rich PPII-helix stretches that due to their structural properties cannot convert to beta-strands and hence disrupt formation of intermolecular beta-sheets.

Abbreviations:

PPII and PPII-helix left-handed polyproline-II type helix.
PrP prion protein.
PrPC normal form of prion protein.
PrPSc pathogenic form of prion protein.
PDB Protein Data Bank.
AD Alzheimer’s disease.
APP amyloid precursor protein.
Abeta  amyloid beta peptide.
NFT  neurofibrillary tangle.
PHF  paired helical filament.

**Author contributions**

AAAd conceived the project, coordinated the study and drafted the paper with contributions from AAM and AAAn. AAAd and AAAn performed computations. AAAd analysed the results with contribution from AAAn. AAM coordinated the study.

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