USING BIOINFORMATICS TO COMPARE THE ROF2 (FKBP65) PROTEIN FROM *ARABIDOPSIS THALIANA* TO ITS HUMAN HOMOLOG FKBP52

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Abstract. Using bioinformatics tools under the ExPasy server, in this paper we made a comparison between the ROF2 protein from *Arabidopsis thaliana* and its human homolog, the FKBP52 protein. The values of the global physicochemical parameters of these proteins are comparable, despite of their low global similarity of the sequences. The first peptidyl prolyl *cis-trans* isomerase domains (PPI1) of the two proteins display the highest sequence similarity. The tetratricopeptide repeats (TPR) involved in protein-protein binding of both proteins also share a relatively high similarity. The results of this study indicate a high structural and functional similarity between the two proteins. Since *Arabidopsis thaliana* is used as a model organism for studying cellular mechanisms involved in human neurodegenerative diseases (which involve FKBP52), our study suggests that ROF2 might play an important role in understanding human neuropathologies.

Key words: immunophilins, FKBPs, ROF2, FKBP65, FKBP52.

INTRODUCTION

The FK506 binding proteins (FKBPs) are present in all organisms and almost all subcellular components. They belong to the peptidyl prolyl *cis-trans* isomerases (PPIases) superfamily [28, 46], which catalyze the isomerisation of the peptide bond between a proline and another residue of a target polypeptide chain, accelerating its folding [22, 41]. The FKBPs are also known as immunophilins, because they bind the immunosuppressive drug FK506 and rapamycin, forming complexes that produce immunosuppression. Binding these drugs, the PPIase activity is inhibited [46].

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Many FKBPs can bind the heat shock protein Hsp90 [56], acting as chaperones by directing the folding of target molecules. The FKBPs are also involved in cell signaling [11], protein trafficking [57], cell division and differentiation [38]. However, most of their functions as well as the mechanisms of their action are unknown.

Using circular dichroism spectroscopy, in our previous work [45] we studied the secondary structure and thermal denaturation of the ROF2 (FKBP65) protein from *Arabidopsis thaliana*. We found that ROF2 presents a remarkable thermal stability, which could be related with its ability to induce thermotolerance [47].

Here we use bioinformatics to compute physicochemical parameters and structural properties of the ROF2 protein, and to compare them with those of the FKBP52 protein (the human homolog of ROF2) with known tertiary structure [68].

The human FKBP52 protein, with molecular mass of 52 kDa, consists of 459 amino acids. It has two PPIase domains: PPI1 (50–138) and PPI2 (167–253), three tetratricopeptide repeats: TPR1 (270–303), TPR2 (319–352), TPR3 (353–386) involved in protein-protein interaction [19], a putative calmodulin-binding region (399–415) [50] and a tubulin-binding domain (267–400) [14].

The ROF2 protein from *Arabidopsis thaliana*, with molecular mass of 65 kDa, consists of 578 amino acids, having three PPIase domains, three tetratricopeptide repeats and a putative calmodulin-binding region [3, 39].

For both proteins, the domain PPI1 is exclusively responsible for the PPIase activity [3, 15]. The TPR domains are responsible for the binding of the heat shock protein Hsp90 [3, 68], which modulates the chaperone activity. It has been shown that both proteins act as modulators of different types of stresses [3, 6, 30, 32, 46] and have the capability to translocate from the cytoplasm to the nucleus, the mechanisms of action being unknown.

FKBP52 is highly expressed in neurons and recent studies revealed that it interacts directly with the microtubule-associated Tau protein (especially with the phosphorylated form of Tau), preventing the filamentous aggregation of Tau in the brain [16, 25], which leads to neurodegenerative diseases, such as the Alzheimer's disease. It has also been shown that FKBP52 protein binds to the transient receptor potential channel 1 (TRPC1) and stimulates its opening (which leads to Ca^{2+} influx), playing a role in the neural chemotropic growth cone and axonal regeneration [58]. The FKBP52 protein also has the capability to interact directly with glucocorticoid receptor (GR) and with the microtubule-associated motor protein dynein [60], being involved in the translocation of the GR complex from the cytoplasm to the nucleus [18, 61] and, probably, in the regulation of nuclear receptors. Therefore, based on its multiple functions in neurons, it is assumed that FKBP52 could play a crucial role in the development of new drugs for the treatment of neuronal diseases.

In order to predict possible structural similarities between FKBP52 and ROF2, as well as the putative capacity of ROF2 to display similar functions to those of FKBP52, we compare the sequences, the physicochemical characteristics and the structural properties of these two proteins.

MATERIALS AND METHODS

The CLUSTAL.0 tool [59] under the ExPasy server (www.expasy.org) was used to perform the sequence alignment and then to compare the sequences of the two proteins.

The PSIPRED tool [37] was used to predict the secondary structure elements of the studied proteins. This program incorporates two feed-forward neural networks that perform an analysis on output obtained from the PSI-BLAST [2], which compares the protein sequences, including distant evolutionary relationships. The secondary structure elements obtained for the ROF2 protein were compared with the known secondary structure elements of FKBP52 protein [68]. In order to estimate the precision of the results, the secondary structure elements of the FKBP52 protein have also been predicted using PSIPRED and compared with the known secondary structure of FKBP52 protein [68].

The ProtParam tool [24] under the ExPasy server (www.expasy.org) was used to compute the global physicochemical parameters of the two proteins: the GRAVY (grand average of hydropathicity) index [40], the aliphatic index [53, 66], the instability index [27], the decadic molar extinction coefficient in water at 280 nm [49] and the theoretical isoelectric point [7].

The ProtScale tool [24] was used to predict the profiles of the secondary structure elements, the hydropathicity and the average flexibility of the proteins under study. The profiles of these properties were computed using various predefined amino acid scales, obtained experimentally, for proteins with known structures. The computations of the profiles use a method of scanning the protein's sequence, with a sliding "window" of a defined length. Each amino acid of the sequence is placed in the centre of the window formed by a certain number of neighbouring amino acids. The average value of the corresponding property, computed over all amino acids of the window, is attributed to the amino acid placed in the centre of the window [40].

For ROF2 and FKBP52, we computed the hydropathicity profile using the Kite-Doolittle hydropathicity scale of the amino acids [40] and the average flexibility profile, using the flexibility indices of the amino acids [5]. For these profiles, we used window lengths of 5, 9 and 13 amino acids, considering that each amino acid of the window contributes with 100% of its predefined value in the chosen scale. We also compared the results obtained using different window sizes.

The transmembrane tendency of ROF2 and FKBP52 has been prospected using several tools: ProtScale [71, 24], TMHMM and HMMTOP [63, 64], TMPred [33] and SOSUI [31].

RESULTS

Figure 1 presents the sequence alignment of the ROF2 (FKBP65) protein (Uniprot ID Q9FJL3) from *Arabidopsis thaliana* and the FKBP52 protein (Uniprot ID Q02790) from *Homo sapiens*, obtained using the CLUSTAL.0 program [59]. The functional domains of FKBP52 are shown above the sequences: the simple and triple straight lines indicate the PPI-ase domains; the simple, double and triple dashed lines indicate the TPR domains [19]; the dot-dashed line indicates the calmodulin binding domain [50]. The double straight line corresponds to the second PPI-ase domain of ROF2, deduced by similarity with the wheat FKBP70 sequence (Uniprot ID Q43207) [48]. The identical positions (*), the conserved substitutions (:) and the semi-conserved substitutions (·) are marked below the sequences. The two sequences have 181 identical positions (the identity being of 181/459 ≈ 40%) and 139 similar positions. The PPI1 domains of the two proteins share the highest identity (of 57/89 ≈ 64%).

Even though the sequence similarity between the two protein sequences is relatively low, the two proteins possess regions with high similarity, which could lead to some other similar functions, besides the known ones (such as the PPI activity, the binding of FK506 and the binding of Hsp90). We further analyze this hypothesis in the discussion section.

Figure 1 also presents the secondary structural elements of FKBP52 protein obtained by Wu *et al.* [68], which are represented below the sequences, with cylinders, arrows and straight lines, according to the legend. The prediction of the secondary structures of ROF2 protein obtained using the PSIPRED tool [37] is presented in Fig.1, too.

In order to evaluate the accuracy of the results, the prediction of the secondary structure of the FKBP52 is also shown in Fig.1, being compared with the known secondary structure of FKBP52 obtained by Wu *et al.* [68]. The net propensity of the two proteins to form α-helices and β-sheets are marked on the sequences, in light and dark gray, respectively. The regions between them are predicted to form random coils. The analysis reveals a good agreement between the known and predicted secondary structure of FKBP52, as well as between the predicted secondary structure elements of ROF2 and FKBP52.

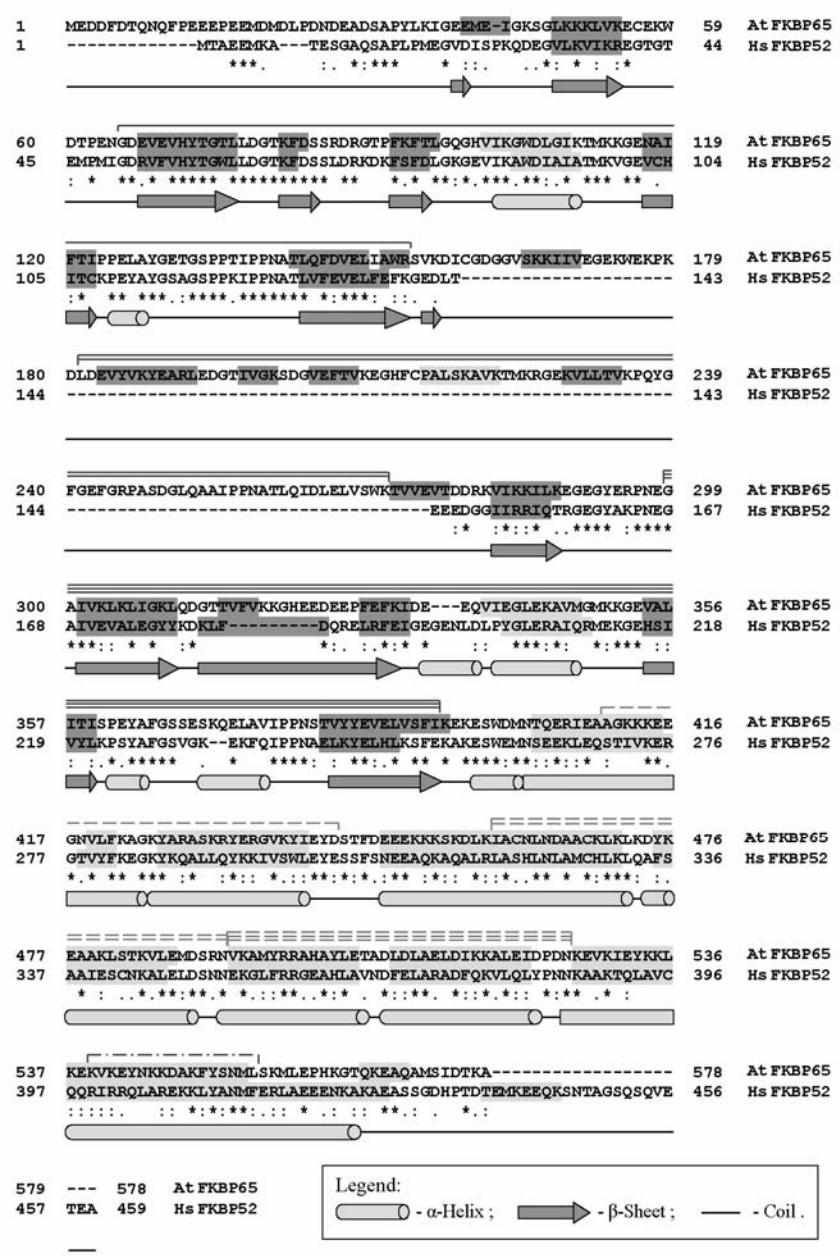

Fig. 1. The sequence alignment obtained using the CLUSTAL.0 tool [59], for the ROF2 protein from *Arabidopsis thaliana* (AtFKBP65) and the FKBP52 protein from *Homo sapiens* (HsFKBP52). The predicted secondary structure elements α-helices and β-sheets obtained using the PSIPRED tool [37] are marked in light and dark gray, respectively. The known secondary structure elements of FKBP52 [68] are represented below the sequences, with cylinders, arrows and lines, according to the legend.

There are a few structural files in the Protein Data Bank (PDB) (http://www.rcsb.org/ – accessed 22 June 2014) [4] concerning the FKBP52 protein. For example, the known secondary structure of FKBP52 protein presented in Fig. 1 has been obtained by Wu *et al*., crystallizing two protein's partially overlapping regions, containing the amino acids 1–260 (PDB ID 1Q1C) and 146–459 (PDB ID 1P5Q), and by constructing a putative tertiary structure of the entire protein [68]. Figure 2 shows the tertiary structures of the two crystallized regions, visualized using the UCSF CHIMERA tool [70]. Other 13 structural files are also available in PDB [4], describing the tertiary structures of other regions of the FKBP52 protein (Uniprot ID Q02790).

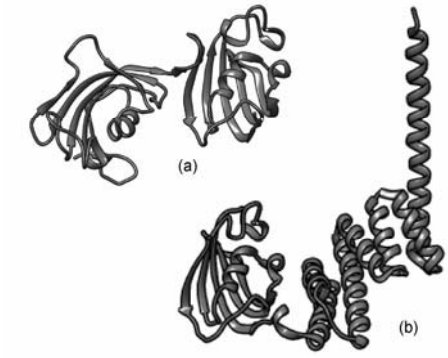

Fig. 2. The tertiary structures of the two partially overlapping regions of FKBP52, crystallized by Wu *et al*. [68], visualized using the UCSF CHIMERA tool [70]: (a) the region containing the amino acids 1–260 (PDB ID 1Q1C); (b) the region containing the amino acids 146–459 (PDB ID 1P5Q).

The physicochemical parameters of ROF2 and FKBP52 are presented in Table 1, where the notations are: N – the number of amino acids in the sequence; M – the molecular mass of the sequence; pI – the theoretical isoelectric point; Q – the net charge; ε_μ – the decadic molar extinction coefficient in water, at 280 nm; $\tau_{1/2}$ – the life-time of the sequence; I – the instability index; A – the aliphatic index; G_H – the GRAVY value.

Most domains of ROF2 and FKBP52 have closely related physicochemical parameters, indicating that the corresponding domains of the two proteins could carry out various similar functions.

Figure 3 presents the hydropathicity profile of the two proteins (Fig. 3, panels a and b), and of their PPI1 domain (Fig. 3, panels c and d), computed using ProtScale [24], with the Kyte&Doolittle hydropathicity scale [40]. The GRAVY values G_H (Table 1) are indicated on the panels. The regions with $G_H > 0$ are considered internal, while those with $G_H < 0$ are considered external [40]. We obtained similar results using the Abraham&Leo [1], Eisenberg *et al*. [21], Janin [35] and Tanford [62] scales (data not shown).

Table 1

The physicochemical parameters of the ROF2 protein from *Arabidopsis thaliana* and the FKBP52 protein from *Homo sapiens*, computed using the ProtParam tool [24] under the ExPasy server

Domains	Protein, amino acids	*N*	*M* (kDa)	pI	*Q*	ε_μ (M^{-1} cm^{-1})	$\tau_{1/2}$	*I*	*A*	G_H
Whole protein	ROF2 1–578	578	65.2232	5.16	–20	63050	30 h – vitro > 20 h – vivo	33.29 (stable)	75.07	–0.702
	FKBP52 1–459	459	51.8045	5.35	–14	46090	30 h – vitro > 20 h – vivo	39.37 (stable)	73.40	–0.643
PPI1	ROF2 65–153	89	9.777	5.21	–3	13980	30 h – vitro > 20 h – vivo	26.56 (stable)	74.49	–0.396
	FKBP52 50–138	89	9.9303	5.60	–2	15595	30 h – vitro > 20 h – vivo	30.44 (stable)	77.75	–0.134
PPI2	ROF2 181–271	92	9.9834	5.81	–1	9970	5.5 h – vitro 3 min – vivo	21.23 (stable)	87.80	–0.227
	FKBP52	–	–	–	–	–	–	–	–	–
PPI3	ROF2 299–393	95	10.521	4.59	–8	4470 (no Trp)	30 h – vitro > 20 h – vivo	45.76 (unstable)	97.37	–0.094
	FKBP52 167–253	87	10.0674	5.18	–4	8940 (no Trp)	30 h – vitro > 20 h – vivo	7.43 (stable)	86.32	–0.546
TRP1	ROF2 410–443	34	3.9535	9.76	+5	5960 (no Trp)	4.4 h – vitro > 20 h – vivo	36.04 (stable)	51.76	–1.256
	FKBP52 270–303	34	4.1268	9.40	+3	11460	1.9 h – vitro > 20 h – vivo	2.99 (stable)	85.88	–0.624
TRP2	ROF2 459–492	34	3.7984	8.73	+2	1615 (no Trp)	20 h – vitro 30 min – vivo	35.53 (stable)	92.06	–0.468
	FKBP52 319–352	34	3.6712	6.01	–1	125 (no Trp)	5.5 h – vitro 3 min – vivo	64.69 (unstable)	109.41	0.182
TRP3	ROF2 493–526	34	3.9024	4.70	–3	2980 (no Trp)	100 h – vitro > 20 h – vivo	23.65 (stable)	106.47	–0.453
	FKBP52 353–386	34	3.9604	6.87	0	1490 (no Trp)	1 h – vitro 30 min – vivo	20.63 (stable)	86.18	–0.594
Calmodulin binding domain	ROF2 539–555	17	2.1064	9.60	+3	2980 (no Trp)	1.3 h – vitro 3 min – vivo	35.83 (stable)	45.88	–1.318
	FKBP52 399–415	17	2.1936	11.57	+5	1490 (no Trp)	1 h – vitro 2 min – vivo	63.37 (unstable)	80.59	–1.012
Tubulin binding domain	ROF2 407–540	134	15.4698	9.10	+6	12045	20 h – vitro 30 min – vivo	39.15 (stable)	80.22	–0.913
	FKBP52 267–400	134	15.2804	8.98	+4	13075	5.5 h – vitro 3 min – vivo	38.49 (stable)	89.70	–0.431

Figure 4 shows the average flexibility profiles of ROF2 (Fig. 4a) and FKBP52 (Fig. 4b), as well as of their PPI1 domains (Fig. 4, panels c, d), performed using ProtScale [24], with the Bhaskaran&Ponnuswamy scale of amino acid flexibility indices [5].

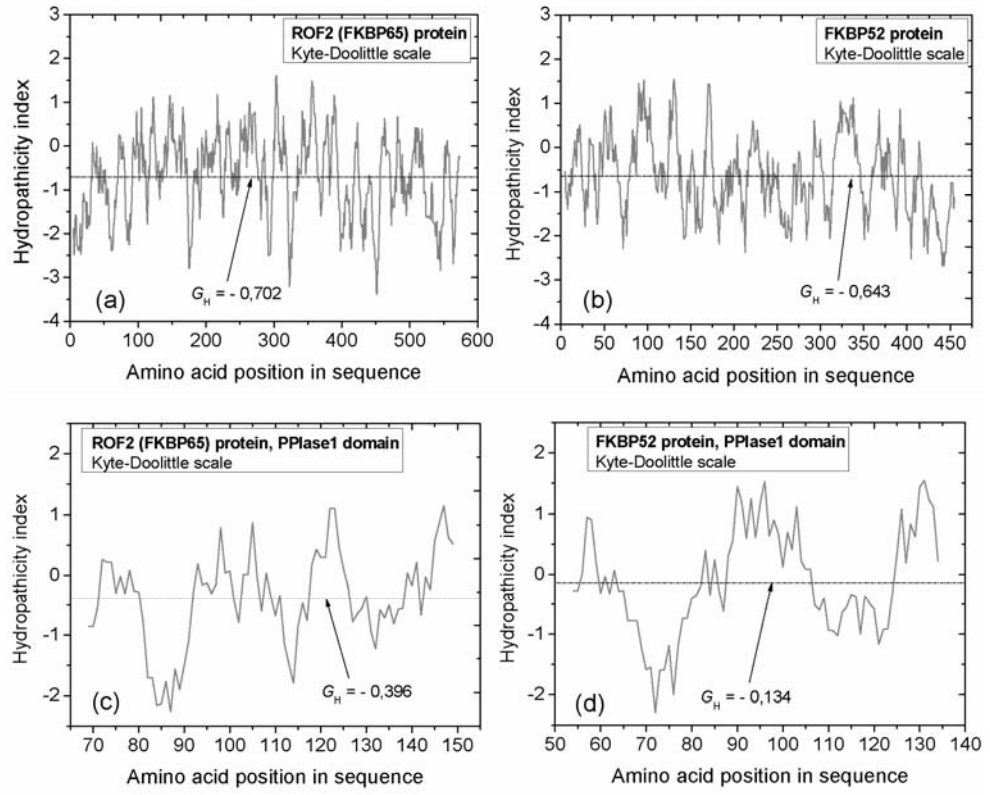

Fig. 3. The hydropathicity profile for ROF2 (a), FKBP52 (b), PPI1 domains of ROF2 (c) and FKBP52 (d). The computations were done using ProtScale [24], with the Kyte & Doolittle hydropathicity scale [40] and a window length of 9 amino acids.

For the entire proteins, we can see short regions of relatively high flexibility that alternate with short regions of lower flexibility.

The PPI1 domain of both proteins presents a middle zone of low flexibility, surrounded by two regions of higher flexibility.

We found similar results for all the computations of the profiles of hydropathicity and flexibility obtained using window lengths of 5 and 13 amino acids, respectively (data not shown).

The investigation of the tendency to anchor or to integrate into membranes indicates that neither ROF2 nor FKBP52 have such a tendency. This result has been consistently obtained by several tools: ProtScale [24, 71], TMHMM, HMMTOP [63, 64], TMPred [33] and SOSUI [31].

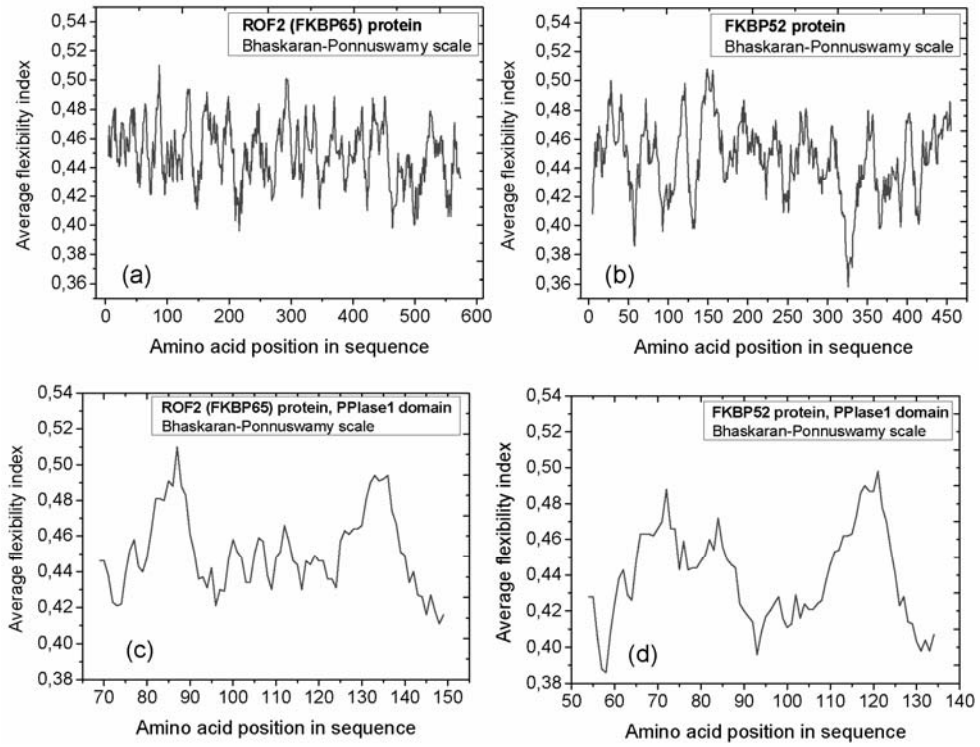

Fig. 4. The average flexibility profiles for ROF2 (a), FKBP52 (b), the PPI1 domain of the ROF2 (c) and FKBP52 (d). The computations have been done using the ProtScale tool [24] and the Bhaskaran & Ponnuswamy flexibility indices of amino acids [5], with a window length of 9 amino acids.

DISCUSSION

THE SEQUENCE ALIGNMENT

The sequence alignment of the ROF2 protein from *Arabidopsis thaliana* (At FKBP65) and the human FKBP52 protein (Hs FKBP52) shows about 40 % identity but there are regions with conserved amino acids and consequently with high similarity (Fig. 1). Our data also reveal a high similarity between the known secondary structural elements of FKBP52 [68] and those predicted using PSIPRED [37] for ROF2 and FKBP52 (Fig. 1), suggesting that we may expect that ROF2 and FKBP52 can carry out certain similar functions. Below, we will analyze further the similarity of some relevant regions of the two proteins.

The highest sequence identity (of about 64%) is observed between the PPI1 domains of the two proteins, with 57 identical positions and 16 similar positions. The PPI1 domains of the two proteins comprise a hydrophobic region of 10

consecutive identical amino acids (76–85 for ROF2 and 61–70 for FKBP52), well conserved in all FKBPs, that form a hydrophobic pocket [65, 57], like a signature of all FKBPs. From this region, the three-dimensional structure of FKBP52 with the bound FK506 (PDB ID 4LAX) [9] indicates that the amino acids Phe67 and Asp68, together with Tyr57, Phe77, Glu85, Val86, Ile87, Trp90, Ala112, Tyr113, Lys121, Phe130, interact with FK506 [9]. From these amino acids, excepting Glu85 and Lys121 which are semi-conserved in ROF2 (the corresponding residues of ROF2 being His100 and Thr136, respectively), all the others are identical with those corresponding of ROF2 (Fig. 1). This result indicates that ROF2 might interact with FK506 just as FKBP52 does.

For FKBP52, the PPI1 domain also has an important role in the interactions with other molecules, being responsible for most of its functions: it is involved in the binding of other PPIases and in the interaction with the microtubule-associated Tau protein [13, 16], playing a possible role in preventing Alzheimer's disease. Also, the PPI1 domain of FKBP52 binds to the microtubule-associated motor protein dynein [60] and, possibly, to the ligand binding domain of steroid hormone receptors [61], being involved in the GR complex formation and its translocation from the cytoplasm to the nucleus. Taking into account that ROF2 also translocates from the cytoplasm to the nucleus [3], as well as the high similarity between the regions around the hydrophobic pocket corresponding to ROF2 and FKBP52 (Fig. 1), we expect that ROF2 could also be involved in similar functions. Even though no nuclear steroid hormone receptors have been found in *Arabidopsis thaliana*, taking into account that the wheat FKBPs can exchange the FKBP52 in steroid GR complex [29], we expect that ROF2 could also be able to replace FKBP52 in complex with GR, Hsp90 and dynein, carrying out certain functions in human neurons. If so, ROF2 could be used for developing new drugs against neurodegenerative diseases.

The PPI1 domain of the two proteins also includes a proline-rich region, comprising the amino acids 123–139 in ROF2 (containing 6 prolines) and 108–124 in FKBP52 (containing 5 prolines, all aligned with those of ROF2). In FKBP52, this region is known to be a loop, responsible for PPIase activity of FKBP52 [43, 68]. It has been shown that some of the FKBP52 protein's functions, such as the binding to the transient receptor potential channel 1 (TRPC1) [58] and, possibly, the binding to microtubules [16] are dependent on the PPIase activity. Due to the high similarity with FKBP52, the corresponding proline-rich region of ROF2 (Fig. 1) seems to be responsible for the PPIase activity of ROF2, as well as for possible PPIase-dependent functions of ROF2.

The three TPR domains of the two proteins, together with the region between TPR1 and TPR2, also display a moderate similarity, with 48 identical positions (identity of about 41 %) and 47 similar positions. The TPR region of FKBP52 is known to bind different target molecules [26], such as the heat shock proteins Hsp90 [61, 68], being necessary for the GR complex formation [52]. Taking into account

the strong similarity between the six known α-helices comprised in the TPR domains of FKBP52 and those predicted for ROF2 and FKBP52, we can expect that the TPR domains of ROF2 could be involved in similar interactions and functions.

The tubulin-binding domains of the two proteins also present a moderate similarity, comprising 51 identical amino acids (identity of 38 %) and 54 similar positions. This observation is consistent with the known involvement of ROF2 in plant growth [3, 47], a process that relies on microtubule activity.

It is known that, in spite of the wide phylogenetic gap between animals and plants, a strong similarity exists between neurons and plant cells [23, 36, 69, 55, 54]. For example, the microtubule-associated proteins (MAPs) are crucial both for neuronal function and for plant growth [23]. In particular, the plant *Arabidopsis thaliana* has a high percentage of genes implied in human diseases, especially in those associated with neuropathologies. Moreover, *Arabidopsis thaliana* presents cellular processes that are similar to those observed in neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease and Huntington's disease), being widely used as a model organism for investigating neuropathologies [36, 55, 54].

The calmodulin-binding domains of the two proteins have a lower similarity, containing 4 identical amino acids (identity of about 24 %) and 9 similar positions.

THE PHYSICOCHEMICAL PARAMETERS OF ROF2 AND FKBP52

Table 1 shows that both ROF2 and FKBP52 proteins have negative net charges (of –20 and –14, respectively), with the theoretical isoelectric points pI = 5.16 for ROF2 and pI = 5.35 for FKBP52.

The instability indices of 33.29 for ROF2 and 39.29 for FKBP52 indicate that the two proteins have a relatively moderate content of dipeptides that are known to produce instability. The lifetimes $\tau_{1/2}$ predict a high stability of both proteins in the test tube, *in vitro*, as well as *in vivo*.

The aliphatic indices of 75.07 for ROF2 and 73.4 for FKBP52 correspond to a high thermal stability of both proteins [34]. This prediction is in agreement with our previous study [45], which, based on circular dichroism experiments, revealed a remarkable thermal stability of ROF2.

The GRAVY values of the two proteins, –0.702 for ROF2 and –0.643 for FKBP52, are lower than the average GRAVY value corresponding to globular soluble proteins (–0.4). These results indicate that ROF2 and FKBP52 have a low net hydrophilic character, which is in good correlation with the physicochemical properties presented above.

The PPIase domains of both proteins are also acidic (Table 1), having a preference for electrostatic interactions with basic, positively charged molecules. The PPI1 domains, which share the highest similarity between the two proteins (Fig. 1), have also similar physicochemical parameters (Table 1). Taking into account that these domains are exclusively responsible for the PPIase activity [51],

the similarity of their properties suggests that ROF2 could be involved in similar PPIase-dependent functions with those of FKBP52.

The highest negative charge of ROF2 ($Q = -8$) corresponds to the third PPI3 domain of this protein (Table 1), aligned with the second PPI domain of FKBP52 (Fig. 1), which has the highest negative charge of FKBP52 ($Q = -4$). In FKBP52, this domain has only a marginal PPIase activity [15].

A remarkable difference between these two PPI domains consists in the difference between their instability indices: 45.76 for the PPI3 domain of ROF2 and 7.43 for the PPI2 domain of FKBP52, indicating a weak instability of ROF2 and a very high stability of FKBP52 in the test tube. Taking into account the lack of one PPI domain in the sequence of the FKBP52 protein, our results might stem from evolutionary changes of FKBP52, which adapted to functions specific to the human organism. Possibly, the PPI domain lost by FKBP52, and present in ROF2, contains the dipeptides that are responsible for the instability of the PPI domain of ROF2.

The TPR1 domains of the two proteins are positively charged. Therefore, they can be involved in the electrostatic binding of acidic, negatively charged molecules. The three-dimensional structure of FKBP52 bound by the heat shock protein Hsp90 is known (PDB ID 1QZ2), showing that FKBP52 interacts with the Hsp90 via the TPR1 domain [68]. On the other hand, it has been found experimentally that ROF2 also interacts with Hsp90 [3]. Taking into account the high similarity of the secondary structural elements of the TPR1 domains corresponding to the two proteins (Fig. 1), we expect that this region of ROF2 is also responsible for the binding of Hsp90.

The TPR3 domain of ROF2 and the TPR2 domain of FKBP52 have extremely high values of the aliphatic indices (106.47 and 109.41, respectively), comparable with those of thermophilic proteins [34], revealing a very high thermal stability of the two proteins. These high values of the aliphatic indices may be related with the hydrophobicity of the aliphatic amino acids, which increases more rapidly when the temperature increases [10, 62]. Therefore, the proteins can remain stable at high temperatures. This property is also in agreement with our previous study [45] and could be related with the possible capability of some FKBPs to act as chaperones *in vitro*, independently on their binding to Hsp90 [8]. The results also correlate well with the detected capability of other PPIases to auto-catalyze their folding [67].

THE HYDROPATHICITY AND FLEXIBILITY OF ROF2 AND FKBP52 PROTEINS

The hydropathicity profiles of the two proteins (Fig. 3 a, b) indicate a rather hydrophilic global character with short, weakly hydrophobic regions alternating with short, hydrophilic regions.

In the PPI1 domains of ROF2 and FKBP52, our results predict a central, low hydrophobic region surrounded by two rather hydrophilic regions (Fig. 3 c, d). The well conserved ten amino acids 61–70 of the FKBP52 and the corresponding amino acids 76–85 of ROF2, which form a hydrophobic pocket [65, 57], are followed by a hydrophilic, external region. The central part of the PPI1 domains (amino acids 92–122 of ROF2 and 77–107 of FKBP52) display low hydrophobicity. This is in agreement with the results of Wu *et al*. [68], which show that FKBP52 contains a substrate binding region, maintained by Trp90 and involved in interactions with other molecules. The proline-rich loops (amino acids 123–139 of ROF2 and 108–124 of FKBP52), responsible for the PPIase activity, correspond to a rather hydrophilic region.

The average flexibility profiles of ROF2 (Fig. 4 a) and FKBP52 (Fig. 4 b) display short regions of relatively high flexibility that alternate with short regions of lower flexibility.

The PPI1 domain of both proteins (Fig. 4 c, d) presents a central zone of low flexibility (corresponding to the binding area surrounded by two flexible regions (corresponding to the binding pocket and to the proline-rich domain).

CONCLUSIONS

In this study, we compared physicochemical and structural properties of the ROF2 protein from *Arabidopsis thaliana* and its human homolog, FKBP52.

In spite of their relatively low global sequence similarity, the secondary structural elements of the two proteins are predicted to be highly similar. Also, their physicochemical parameters generally have close values, suggesting that they could carry out various similar functions.

As expected, the PPI1 domains of ROF2 and FKBP52 share the highest similarity of their sequences, secondary structural elements and physicochemical properties.

The TPR domains of the two proteins also have a high sequence similarity and a strong similarity of the secondary structural elements. Therefore, we expect that they could be involved in similar interactions. The TPR3 domain of ROF2 and the TPR2 domain of FKBP52 have extremely high values of the aliphatic indices. This property could explain the ability of ROF2 to accomplish its functions at high temperatures, which trigger its synthesis.

As expected, the ROF2 and FKBP52, as well as their major domains, are predicted to be stable, in agreement with their known ability to modulate different types of stress. Their aliphatic indices are high, revealing a high thermal stability of the two proteins, in agreement with our previous results [45]. The GRAVY values of the two proteins indicate a rather hydrophilic global character.

In both proteins, the PPI1 domain includes a central, flexible region with a low hydrophobic character, which could constitute an interacting area surrounded by two flexible regions, which could also be involved in the interactions with other proteins. Such a flexibility profile might be responsible for the PPIase activity.

This study illustrates the usefulness of structural bioinformatics tools for protein structure and function predictions.

Taken together, the results presented here indicate a high structural and functional similarity of ROF2 and FKBP52.

Taking into account that *Arabidopsis thaliana* is currently viewed as a model organism that displays phenomena observed in human neuropathologies [36, 55], we expect that ROF2 might play an important role in the development of new drugs for neurodegenerative diseases.

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