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Unique Proline-Rich Domain Regulates the Chaperone Function of AIPL1

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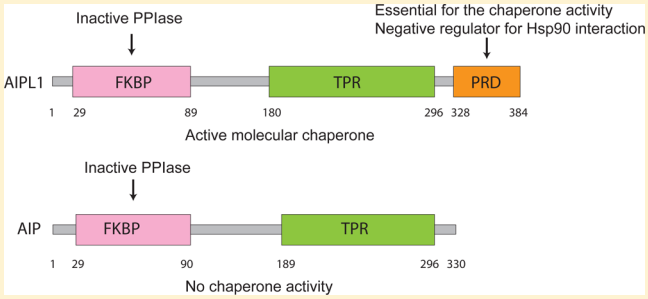
Supporting Information

ABSTRACT: Human aryl hydrocarbon receptor (AHR) interacting protein (AIP) and AIP like 1 (AIPL1) are cochaperones of Hsp90 which share 49% sequence identity. Both proteins contain an N-terminal FKBP-like prolyl peptidyl isomerase (PPIase) domain followed by a tetratricopeptide repeat (TPR) domain. In addition, AIPL1 harbors a unique C-terminal proline-rich domain (PRD). Little is known about the functional relevance of the individual domains and how these contribute to the association with Hsp90. In this study, we show that these cochaperones differ from other Hsp90-associated PPIase as their FKBP domains are enzymatically inactive. Furthermore, in contrast to other large PPIases, AIP is inactive as a chaperone. AIPL1, however, exhibits chaperone activity and prevents the aggregation of non-native proteins. The unique proline-rich domain of AIPL1 is important for its chaperone function as its truncation severely affects the ability of AIPL1 to bind non-native proteins. Furthermore, the proline-rich domain decreased the affinity of AIPL1 for Hsp90, implying that this domain acts as a negative regulator of the Hsp90 interaction besides being necessary for efficient binding of AIPL1 to non-native proteins.



INTRODUCTION

The heat shock protein 90 (Hsp90) is an abundant molecular chaperone which is essential in eukaryotic cells.[1−5] Hsp90 acts at the interface between pathways of protein folding and conformational regulation. It plays a prominent role in many human diseases such as cancer.[6] In this context, its interaction with signal-transduction proteins seems to be of key importance.[3] Over the last decades, many Hsp90 cochaperones have been discovered and characterized.[7−10] It is thought that some of the cochaperones facilitate the binding of Hsp90 to client proteins and confer specificity to this process.[11−14] Others are implicated in regulating the conformational cycle of Hsp90.[15−19]

For Hsp90 clients such as transcription factors and steroid hormone receptors (SHRs), it has been shown that a number of cochaperones are required together with Hsp90 for maintaining an activatable state.[8,20−22] In the case of the aryl hydrocarbon receptor (AHR), a trimeric complex consisting of the receptor, Hsp90, and the cochaperone AIP (aryl hydrocarbon receptor interacting protein, also called Xap2 or FKBP37) had been identified.[23] The cochaperone AIP seems to play an essential role in the regulation of AHR activity.[24,25] Recent studies indicated that AIP negatively regulates the function of different SHRs.[26,27] Moreover, mutations in AIP are connected with a predisposition to pituitary adenomas.[28] AIP is closely related to the AIP-like protein 1 (AIPL1), which was originally identified in the context of the autosomal recessive eye disease Leber's congenital amaurosis (LCA).[29] LCA is the most rapid and severe form of congenital blindness, and AIPL1 mutations result in the most severe forms of LCA.[30] Human AIPL1, as well as AIP, contain an N-terminal prolyl-peptidyl-isomerase (PPIase) domain and a tetratricopeptide repeat (TPR) domain. They share partial sequence homology with the well-studied PPIases FKBP52 and FKBP51, which are found in complexes together with Hsp90 and steroid hormone receptors.[31,32] PPIases are a large class of proteins that catalyze the *cis/trans* isomerization of prolyl peptide bonds.[33]

Here, we performed a structure−function analysis of AIP and AIPL1. We show that both AIP and AIPL1 are inactive as PPIases. However, AIPL1, but not AIP, shows chaperone activity, and this function is mediated by the proline-rich domain.

MATERIALS AND METHODS

Protein Purification. AIP and AIPL1 were purified as described previously.[34,35] The construct AIPL1−ΔPRD, lacking the proline-rich domain, was created by PCR. The proline-rich domain was inserted into AIP by PCR to generate the AIP−

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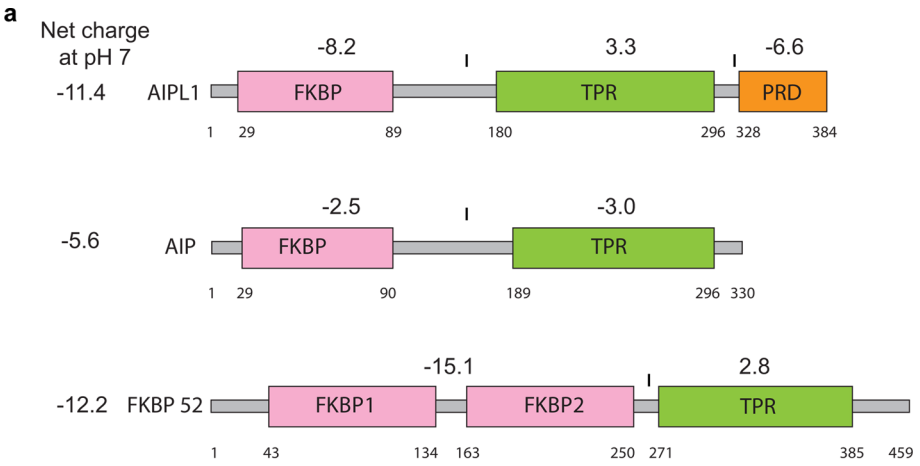

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AIP       MADIIARLREDGIQKRVIQEGRGELPDFQDGTKATFHYRTLHSDDEGTVLDDSRARGKPM
AIPL1     -MDAALLLNVEGVKKTILHGGTGELPNFITGSRVIFHFRTMKCDEERTVIDDSRQVGQPM
            *    *. :*::* ::: * ****:*  *::. **:**::.*:* **:****  *:**

AIP       ELIIGKKFKLPVWETIVCTMREGEIAQFLCDIKHVVLYPLVAKSLRNIAVGKDPLEGQRH
AIPL1     HIIIGNMFKLEVWEILLTSMRVHEVAEFWCDTIHTGVYPILSRSLRQMAQGKDPTEWHVH
          .:***: *** *** :: :**  *:*:* **  *. :**::::***::* **** * : *

AIP       CCGVAQMREHSSLGHADLDALQQNPQPLIFHMEMLKVESPGTYQQDPWAMTDEEKAKAVP
AIPL1     TCGLANMFAYHTLGYEDLDELQKEPQPLVFVIELLQVDAPSDYQRETWNLSNHEKMKAVP
           **:*:*  : :**: *** **::****:* :*:*:*::*. **::.* :::.** ****

AIP       LIHQEGNRLYREGHVKEAAAKYYDAIACLKNLQMKEQPGSPEWIQLDQQITPLLLNYCQC
AIPL1     VLHGEGNRLFKLGRYEEASSKYQEAIICLRNLQTKEKPWEVQWLKLEKMINTLILNYCQC
          ::* *****:: *: :**::** :** **:*** **:* . :*::*:: *..*:******

AIP       KLVVEEYYEVLDHCSSILNKYDDNVKAYFKRGKAHAAVWNAQEAQADFAKVLELDPALAP
AIPL1     LLKKEEYYEVLEHTSDILRHHPGIVKAYYVRARAHAEVWNEAEAKADLQKVLELEPSMQK
           *  *******:* *.**.:: . ****: *.:*** ***  **:**: *****:*::

AIP       VVSRELQALEARIRQKDEEDKARFRGIFSH------------------------------
AIPL1     AVRRELRLLENRMAEKQEEERLRCRNMLSQGATQPPAEPPTEPPAQSSTEPPAEPPTAPS
          .* ***: ** *: :*:**:: * *.::*:

AIP       -------------------------
AIPL1     AELSAGPPAEPATEPPPSPGHSLQH
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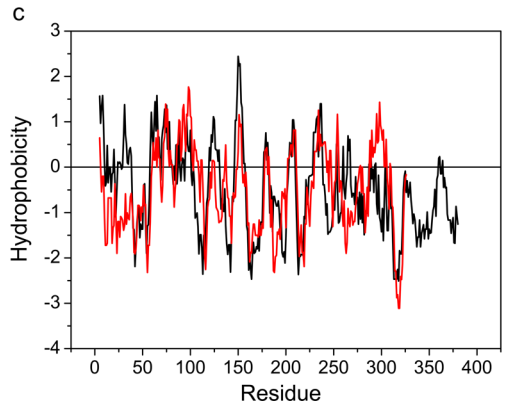

Figure 1. Domain architecture and sequence alignment of AIP and AIPL1. (a) Domain organization of AIPL1, AIP, and FKBP52. AIPL1 contains one FKBP-like domain, followed by a TPR domain and a unique C-terminal proline-rich domain (PRD). AIP contains one FKBP-like domain and a TPR domain. FKBP52 contains two FKBP domains and one TPR domain. The amino acid positions are indicated by numbers. Protein net charges at pH 7.0 were analyzed by Protein Calculator v.3.3 (http://www.scripps.edu/~cdputnam/protcalc.html) (b) Sequence alignment of AIP and AIPL1. (*) indicates identical or conserved residues in all sequences in the alignment, (:) implies conserved substitutions, and (.) highlights semiconserved substitutions. (c) Kyte−Doolittle hydropathy plots of AIP (red) and AIPL1 (black). The hydrophobicity of AIP and AIPL1 are analyzed by ProtScale (http://web.expasy.org/protscale/) using the Kyte−Doolittle algorithm.

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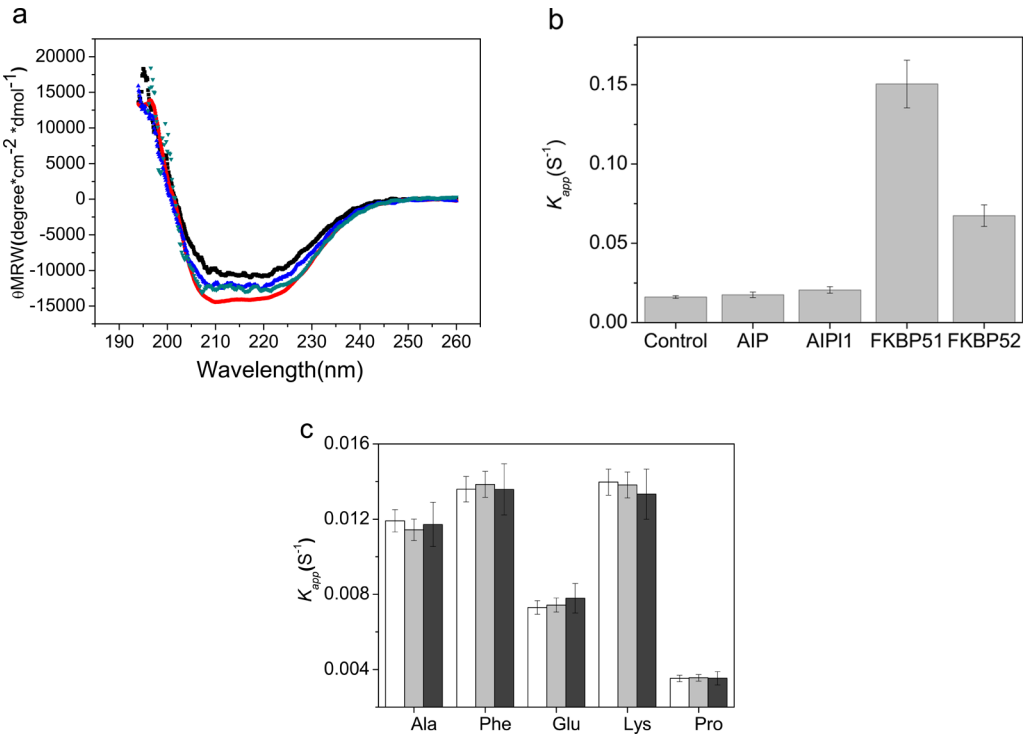

Figure 2. Structure and PPIase activity of AIP, AIPL1, AIP−-PRD, and AIPL1ΔPRD. (a) Far UV CD spectra of AIP (black), AIPL1 (blue), AIP−PRD (turquoise), and AIPL1−ΔPRD (red) measured at 20 °C. (b) PPIase activity was measured using a fluorescent peptide-based method.[36] Tyr (aminobenzoyl-Ala-Xaa-Pro-Phe-4-nitroanilide(Abz-Ala-Xaa-Pro- Phe-pNA)) peptides (2 μM) were used in the assays. The measurements were performed at 15 °C in a buffer containing 40 mM HEPES/KOH pH7.5, 100 mM KCl, and 1 mM DTT in the presence of 1 μM of proteins. As a control, the peptide in the absence of any additional components was measured. To examine the PPIase activity of AIP and AIPL1, the isomerization of different peptides was measured in the absence (white) or presence of 1 μM AIP (light gray) or AIPL1 (dark gray) (c).

PRD variant. A plasmid of wild-type AIPL1 [34] served as template, and the product was cloned into the plasmid pET28b resulting in the addition of an N-terminal His-tag. The correct sequence of positive clones was confirmed.

To purify AIPL1−ΔPRD and AIP−PRD, Rosetta cells were transformed with the plasmid and grown at 37 °C in Luria−Bertani broth containing 35 μg/mL kanamycin to an optical density of about 0.6 at 600 nm. Then, the cells were induced with IPTG (final concentration 1 mM) overnight at 30 °C. Cells were harvested, disrupted and centrifuged. AIPL1−ΔPRD was purified from the soluble franction by NiNTA affinity chromatography, anion-exchange chromatography using a Resource Q column, and size-exclusion chromatography on a Superdex 200 prep grade column (GE Healthcare, Munich, Germany).

CD spectroscopy. CD spectroscopy was performed in a JASCO J-715 spectropolarimeter (JASCO, Groß-Umstadt, Germany) equipped with a PTC 343 peltier unit. Proteins with a concentration of 0.1 mg/mL were measured in 10 mM sodium phosphate buffer, pH 7.5, if not indicated otherwise. To determine the thermal stability of AIP, AIP−PRD, AIPL1, and AIPL1−ΔPRD, the CD signal of both proteins was monitored at 220 nm from 20 to 80 °C with a heating rate of 20 °C/h.

Prolyl-isomerase Assay. The assay was performed as described previously.[36] Briefly, peptides (Abz-Ala-Xaa-Pro-Phe-pNA) were dissolved in anhydrous 0.55 M LiCl/TFE mixture. Solvent jumps were initiated by addition of the 2 μL aliquot of the 1 mM peptide stock solution to 998 μL of 40 mM HEPES, 100 mM KCl, 1 mM EDTA, pH 7.5 in a fluorescence cell in the absence or presence of PPIases. Final peptide concentration was 2 μM. Fluorescence at 416 nm was detected after excitation at 316 nm using a JASCO FP 6500 spectrofluorimeter. The time courses of the *cis*−*trans* isomerization were analyzed as single-exponential reactions.

Chaperone Assay. Citrate synthase (CS) (0.5 μM) was thermally denatured by incubation at 43 °C in 40 mM HEPES/KOH pH 7.5 for 40 min. Aggregation of non-native CS was measured by monitoring the increase of turbidity at 360 nm in a Cary 50 UV−vis spectrophotometer (Varian, Palo Alto, CA) equipped with a temperature control unit using microcurets with a path length of 1 cm. Thermal inactivation of CS was performed as described with a CS concentration of 0.15 μM.[37]

ANS Binding Assay. To study binding of the hydrophobic probe, bis-ANS, 15 μM of the wild type or chimeric proteins in 40 mM HEPES/KOH buffer, pH 7.5 containing 100 mM KCl were used. To each of the protein samples (1.2 mL) was added 15 μL of 0.8 mM methanolic solution of bis-ANS, and the samples were incubated for 10 min at 37 °C. Fluorescence spectra of the samples were recorded from 400 to 650 nm with the excitation wavelength set at 390 nm.

SPR Spectroscopy. SPR experiments were performed with a Biacore X Instrument. A CM5 chip (GE Healthcare, Munich, Germany) was coupled with human Hsp90 using amine coupling reagents, yielding 1000 resonance units (RU) of immobilized protein. Measurements were performed at 25 °C in 40 mM HEPES/KOH pH 7.5, 50 mM KCl at a flow rate of 20 μL/min. Injections (65 μL) of different protein concentrations were performed. Plateau values during binding reactions were determined and plotted against the concentration of injected protein. The corresponding curves were analyzed based on plateau values during the injection using the Origin software (OriginLab Corp, Northampton, MA).

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RESULTS

AIP and AIPL1 Show High Sequence and Structural Similarity but a Distinct Difference in the C-Terminal Region. AIP and its homologue AIPL1 share 49% sequence identity (Figure 1a,b). Both proteins have an N-terminal FKBP-like domain, which suggests that AIP and AIPL1 might possess PPIase activity, like FKBP12.[38] The conserved TPR domain seems to be involved in Hsp90 interaction[15] (Figure 1a,b). The C-terminal regions of AIP and AIPL1 are strikingly different. AIPL1 contains a unique proline-rich domain with XXPP repeats in its very C-terminal region (Figure 1b). This proline-rich region is present only in primates and shows considerable sequence variation.[39] Similar to the well-studied PPIase FKBP52, AIPL1 has a high negative net charge, which is known to increase protein solubility and commonly found in chaperones like Hsp90, Hsp70, and Hsp33.[40] The negative charges are localized in the FKBP-like domain and the proline-rich region (Figure.1a). In addition, we performed a hydrophobicity analysis of AIP and AIPL1. The results revealed different hydrophobicity patterns (Figure 1c). AIPL1 has two hydrophobic patches located near the N-terminus and the linker region between the FKBP-like domain and the TPR domain, while the C-terminal proline-rich region is hydrophilic.

To address their structural and enzymatic properties, we expressed and purified AIP and AIPL1. As shown by far-UV CD spectroscopy, both proteins have an ordered structure with strong signals at 208 and 222 nm, indicating a high α-helical content (Figure 2a). AIP and AIPL1 also exhibit similar protein stability. The midpoint of the thermal transitions, as measured by CD spectroscopy, was around 45 °C for both proteins (Table 1 and Supplementary Figure 1, Supporting Information). To analyze effects of the unique proline-rich domain on the structure and function of AIPL1, we created an AIPL1 variant lacking the proline-rich domain (AIPL1-ΔPRD). In addition, a chimeric protein consisting of AIP and the proline-rich domain from AIPL1 (AIP-PRD) was constructed and purified. Similar to the wild-type protein, both variants show a typical α-helical signature (Figure 2a). The melting temperature is 44 °C for AIPL1-ΔPRD and 49 °C for AIP-PRD (Table 1). Based on these experiments, we conclude that the proline-rich domain does not impact the structural properties and thermal stability of AIPL1.

Table 1. Thermal Stability and Hsp90 Binding Constants of AIP, AIPL1, and Their Variants[a]

	AIP	AIP-PRD	AIPL1	AIPL1-ΔPRD
T_m (°C)	46.7 ± 0.4	49.3 ± 0.2	44.1 ± 0.2	43.9 ± 0.5
K_D (μM)	2.28	6.88	2.60	0.89

[a]To assess the stability of the different proteins, temperature-induced unfolding experiments were performed. Temperature-induced unfolding was monitored by far UV−CD spectroscopy at a fixed wavelength with a heating rate of 20 °C/h. Data were fitted to obtain transition midpoints. The affinities of AIP, AIPL1, and their variants to Hsp90 were determined by SPR. Each K_D value was calculated on the basis of injections with different concentrations of protein onto a human Hsp90-coated CM5 chip.

AIP and AIPL1 Are inactive PPIases with Altered Function. AIP and AIPL1 share homology to the PPIase domain of FKBPs. To test whether AIP and AIPL1 catalyze peptidyl-prolyl isomerization in vitro, we performed protease-free fluorescence-based PPIase assays.[36] As a control, we used FKBP51 and FKBP52, Hsp90-associated PPIases with reported prolyl-isomerization activity.[41]

First, PPIase activities were measured using an amino-benzoyl-Ala-Tyr-Pro-Phe-4-nitroanilide model peptide. In the absence of PPIases, the *cis*/*trans* isomerization rate was 0.014 s^{-1}. In the presence of 1 μM FKBP51 or FKBP52, the isomerization reaction of the peptide was accelerated 10- or 5-fold, respectively (Figure 2b). No influence on the isomerization rate was however observed in the presence of 1 μM AIP or AIPL1 (Figure 2b). FKBPs are very specific regarding the residues preceding the Xaa-proline (P1 position) and they have the highest affinity for aromatic, hydrophobic residues.[42] To investigate whether AIP or AIPL1 display a different recognition pattern, the isomerization of other proline-containing peptides with a different P1-residue was investigated for Ala, Phe, Glu, Lys or Pro. Catalysis of isomerization was not observed for any of these peptides (Figure 2c). We conclude that both AIP and AIPL1 are inactive as PPIases under the conditions tested.

AIPL1 but not AIP Shows Molecular Chaperone Activity. In addition to PPIase activity, FKBP52 and 51 also exhibit chaperone activity.[43] To analyze whether AIP and AIPL1 interact with an unfolding protein, we performed the well-established citrate synthase (CS) aggregation and inactivation assays.[37] CS loses its activity and aggregates rapidly when incubated at 43 °C, which can be followed by monitoring the turbidity of the protein solution. In these experiments, AIP did not influence the temperature-induced aggregation of CS, even when added in large excess (Figure 3a). Surprisingly, AIPL1 showed strong chaperone activity. A 3-fold excess of AIPL1 suppressed the thermal aggregation of CS completely, and an equal molar ratio was sufficient for half-maximum suppression (Figure 3a). Comparison of the insoluble pellet fraction and the soluble supernatant fraction by SDS-PAGE supports the light-scattering results (Figure 3b). In the presence of increasing concentrations of AIPL1, increasing amounts of CS remained soluble at 43 °C.

Next, we aimed to determine whether AIPL1 protects CS from thermal inactivation. To this end, we measured CS activity after different time points of incubation at elevated temperatures (43 °C.). The results indicate that AIPL1 slowed down the thermal inactivation of CS while AIP had no effect (Figure.3c). However, after 30 min of heat shock, the remaining activity of CS was less than 10% of the native protein, even though light scattering experiments indicated that CS remained in a soluble form (Figure 3c). Taken together, AIPL1 seems to act as a holdase which is capable of maintaining CS in a nonaggregated form.

Proline-Rich Domain Is Critical for the Chaperone Activity of AIPL1. As AIPL1, but not AIP, binds specifically to non-native proteins, we tested whether the C-terminal proline-rich domain affects the interaction of AIPL1 with unfolded substrates. In contrast to wild type AIPL1, the presence of the C-terminally truncated mutant AIPL1-ΔPRD did not have any influence on the aggregation behavior of CS, even when present in large excess (Figure 4a). Similarly, no effects could be observed in the CS inactivation assay in the presence of AIPL1−ΔPRD (Figure 4b). These data suggest that the C-terminal proline-rich region is required for the chaperone function of AIPL1.

Next, we asked whether the function of the proline-rich domain could be transferred to AIP. To this end, we created the chimeric protein AIP−PRD, in which the proline-rich domain

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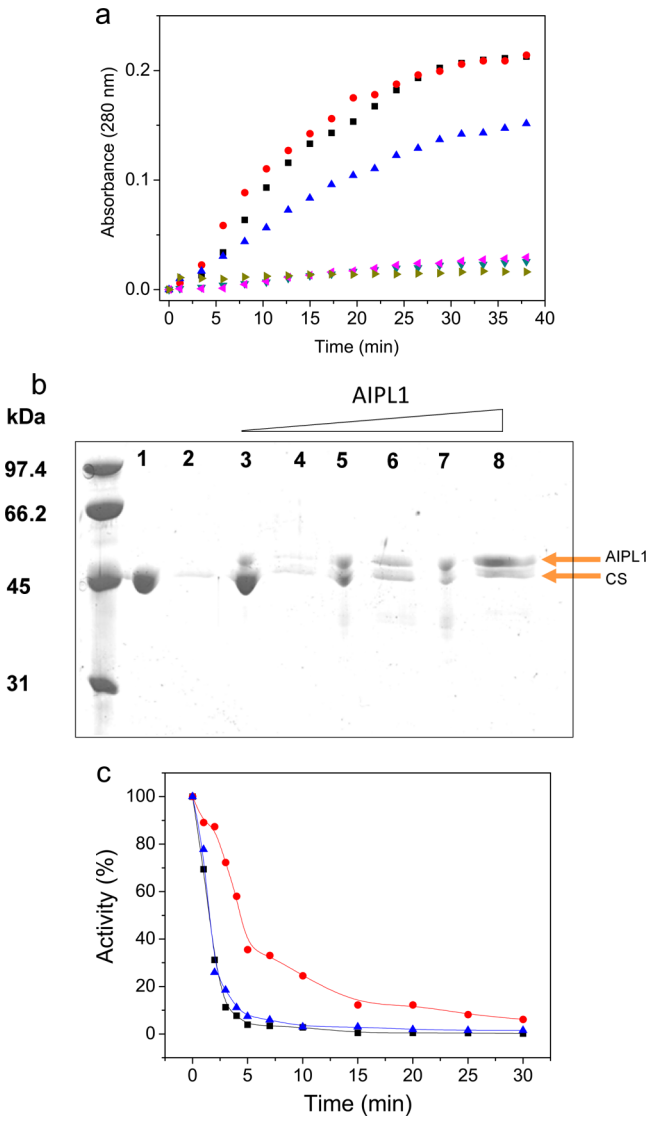

Figure 3. AIPL1 is a molecular chaperone. (a) Influence of AIP and AIPL1 on the aggregation of thermally denatured CS. CS (0.5 μM) were incubated at 43 °C in 40 mM HEPES/KOH, pH 7.5. CS aggregation was monitored by measuring the absorption at 360 nm in the absence of additional components (black) or in the presence of an equal molar amount (blue), a 3-fold molar excess (pink), a 5-fold molar excess (green), or a 10-fold molar excess (dark yellow) of AIPL1. As a control, a 10-fold molar excess of AIP was added to CS (red). (b) AIP prevents the thermal aggregation of CS. CS (0.15 μM) was incubated alone or together with different concentration (1:1, 3:1, and 5:1) of AIPL1 at 43 °C for 30 min. The soluble supernatant and the aggregates were subjected to SDS analysis. The orange arrows indicate the size of CS and AIPL1. Lanes 1, 3, 5, 7: pellet; lanes 2, 4, 6, 8: supernatant. (c) AIPL1 slows down the thermal inactivation of a model client protein. Inactivation kinetics of citrate synthase (0.15 μM) were recorded following incubation at 43 °C (black). The effect of AIP (blue) and AIPL1 (red) on the inactivation kinetics was measured in the presence of a 5-fold molar excess of AIP or AIPL1 over CS.

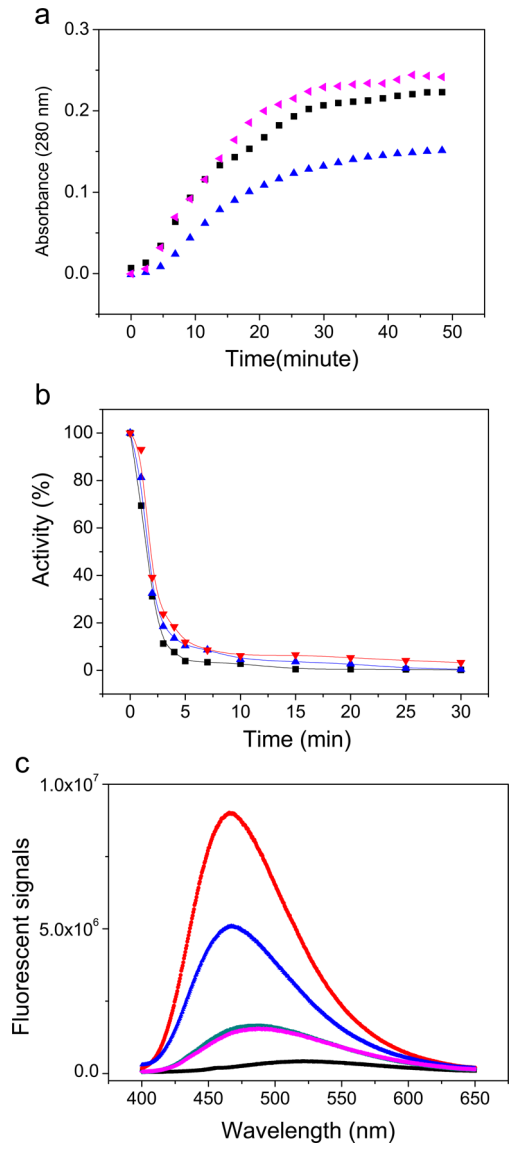

Figure 4. Proline-rich domain is necessary but not sufficient for the chaperone activity. (a) Influence of AIP−PRD (blue) and AIPL1−ΔPRD (pink) on aggregation of thermally denatured CS. CS sample (0.5 μM) was incubated at 43 °C in 40 mM HEPES/KOH, pH 7.5. CS aggregation was monitored by measuring the absorption at 360 nm in the absence of additional components (black) or in the presence of a five-fold molar excess of AIP−PRD (blue) or AIPL1−ΔPRD (pink). (b) Inactivation kinetics of CS (0.15 μM) (black) were recorded following incubation at 43 °C. The effect of AIP−PRD (blue) and AIPL1−ΔPRD (red) on the inactivation kinetic was measured with five-fold molar excess of AIP−PRD over CS. (c) AIP, AIPL1, and their variants differ in their hydrophobic surfaces. Fluorescence emission spectra of ANS (100 μm, in HEPES/KOH, 50 mM KCl, pH 7.5) were measured in the presence of AIP (turquoise), AIPL1 (red), AIP-PRD (pink), or AIPL1-ΔPRD (blue). As a control, fluorescence spectra for ANS were recorded for the dye alone (black) after excitation at 395 nm.

was fused to AIP. Indeed, suppression of CS aggregation was achieved in the presence of an excess of AIP−PRD (Figure 4a). However, compared to wild-type AIPL1, AIP−PRD showed reduced chaperone activity and was unable to protect CS from thermal inactivation (Figure 4b). Taken together, the C-terminal proline-rich region is necessary but not sufficient for the chaperone function of AIPL1.

Since the interaction between the chaperones and a client protein often involves hydrophobic interactions, we tested the accessibility of hydrophobic patches using the probe bis-ANS. This dye binds much better to AIPL1 than to AIP, suggesting larger hydrophobic surfaces on AIPL1 (Figure 4c). Deletion of

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the proline-rich domain resulted in a dramatic decrease of the bis-ANS fluorescence intensity. This observation parallels the results that AIPL1 exhibits higher chaperone activity compared to AIPL1-ΔPRD.

Proline-Rich Domain Is a Negative Regulator of Hsp90 Interaction. AIP is known to form a ternary complex with Hsp90 and AHR.[25] Furthermore, AIPL1 was recently shown to be part of a chaperone heterocomplex together with Hsp70/Hsp90,[44] consistent with the notion that both proteins are potential Hsp90 cochaperones. However, the binding of either AIP or AIPL1 to Hsp90 had not been characterized in vitro yet. To address this question, we determined the relative affinities of these proteins for Hsp90 by surface plasmon resonance (SPR) with human Hsp90 covalently coupled to the chip surface. We found that both AIP and AIPL1 bind to Hsp90 with similar dissociation constants of 2 μM (Table 1 and Supplementary Figure 2, Supporting Information). These affinities are much weaker than the affinities between Hsp90 and FKBP51 or FKBP52.[41] Surprisingly, the affinity of AIPL1−ΔPRD for Hsp90 was increased when compared to the full-length AIPL1 (Table 1 and Supplementary Figure 2, Supporting Information, 0.9 μM versus 2.6 μM). In contrast, the binding constant of AIP−PRD increased from 2.3 to 6.9 μM for AIP upon addition of the proline-rich domain, implying that this domain influences the interaction with Hsp90 (Table 1 and Supplementary Figure 2, Supporting Information). Thus, besides the chaperone activity, the C-terminal proline-rich region of AIPL1 also has the potential to influence the association with Hsp90 in an inhibitory manner.

DISCUSSION

AIP and AIPL1 have highly similar primary structures but distinct biological functions. AIP is ubiquitously expressed in almost all tissues, and variants of AIP are associated with familial pituitary adenomas.[45,46] In contrast, AIPL1 is expressed specifically in adult rod photoreceptor cells, where its function is essential but not well understood.[47]

Except for a unique proline-rich domain in the C-terminal part of AIPL1, both proteins have a similar domain organization in which an N-terminal PPIase-like domain is followed by a TPR domain composed of three consecutive TPR motifs. In this study, we demonstrate that both AIP and AIPL1 are inactive PPIases, not able to catalyze the cis−trans isomerization of proline-containing peptides in vitro. While the assays mentioned in this study rule out a general PPIase activity as determined for other members of this family,[48] it might be that these proteins are active in vivo only for very specific substrates. A unique helix and a long insert between the last two strands in the FKBP domain of AIP, as shown in the structure of this domain by Linnert et al., (cosubmitted study[49]) seem to be important elements for the binding specificity of AIP. In general, PPIase-containing TPR proteins during the maturation of SHR may be selected by specific client proteins. Cyp40, for example, is most abundant in estrogen receptor (ER) complexes[50] and FKBP52 mediates potentiation of glucocorticoid receptors (GR) but not estrogen receptors.[32] Interestingly, the PPIase activity of FKBP52 was found to be not required for the maturation of SHRs, as mutations lacking the isomerase activity did not affect potentiation.[51] Therefore, the PPIase domain of AIP and AIPL1 may also possess a noncatalytic function. In this context, a recent study on the Hsp90 chaperone cycle indicated that AIP could form a ternary complex with Hsp90 and the cochaperone Hop, which facilitates the progression of the cycle.[52]

Moreover, we show here that only AIPL1, but not AIP, is a chaperone. This is reminiscent of the Hsp90 associated PPIases FKBP51 and -52 and Cyp40.[41,43,53] Interestingly, for FKBP52 it could be shown that the linker region connecting the FKBP domain and TPR domain plays an important role in the chaperone activity.[54] The involvement of a proline-rich domain in chaperone functions is so far unique. It is well-known that many molecular chaperones prevent the aggregation of misfolded proteins by binding to hydrophobic patches.[55] The hydrophobic patches in the N-terminus and the linker region between FKBP-like domain and TPR domain may contribute to the chaperone activity of AIPL1. However, the C-terminal proline-rich region is essentially hydrophilic. Nevertheless, this charge-rich region greatly influence the chaperone activity, in agreement with previous studies.[40] Thus, the chaperone activity of AIPL1 seems to require the cooperation between the proline-rich domain and other domains in AIPL1, as efficient chaperone activity was only observed for the authentic protein and the fusion of the proline-rich domain to AIP resulted in a modest gain in chaperone activity. Proline-rich regions are common modules mediating protein−protein interactions, especially in proteins involved in signaling transduction pathways, like p53,[56] and a number of different consensus motifs have been described.[57] While exhibiting general chaperone activity in vitro, the unique pattern of the proline-rich domain of AIPL1 may be correlated to the chaperoning of specific client proteins. One potential client of AIPL1 is NEDD8 ultimate buster 1 (NUB1), which recruits the ubiquitin-like protein NEDD8 and its conjugates for proteasomal degradation.[58] AIPL1 was found to suppress the aggregation of NUB1 in vivo and modulate its nuclear translocation.[59] AIPL1 is also essential for the assembly of the rod phosphodiesterase PDE6, a critical tetrameric enzyme which is needed to convert light stimuli into electrical responses in the vision process.[60] Since AIPL1 interacts with Hsp90, also the complex may be involved in the quality control processes and the proper assembly of PDE6.

Our study expands the concept of the functions of TPR-containing large PPIases associating with Hsp90. It remains to be seem how the specific features described here and in previous studies modulate Hsp90 client complexes.

ASSOCIATED CONTENT

Supporting Information

To assess the stability of AIP, AIPL1 and their variants, temperature-induced unfolding experiments were performed (Figure S1). Data were fitted to a Boltzmann function to obtain transition midpoints. The binding constant between AIP, AIPL1 or their variants and Hsp90 was determined by SPR titrations (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

J.L. designed and performed the experiments, analyzed the data, and wrote the manuscript. G.Z. performed and analyzed the PPIase assay. J.S. performed yeast assays to analyze the function of AIP and AIPL1. T.K. performed mass spectrometry experiments to analyze the interaction partners for AIP and AIPL1. K.R. contributed to writing the manuscript. J.B. and F.X.S. supervised the experiments and wrote the manuscript.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PRD: proline-rich domain; AHR: aryl hydrocarbon receptor; PPIase: prolyl-peptidyl-isomerase; AIP: aryl hydrocarbon receptor interacting protein; AIPL1: AIP like 1; LCA: Leber's congenital amaurosis; TPR: tetratricopeptide repeat; SPR: surface plasmon resonance; SHR: steroid hormone receptor

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