Peptide aptamers: tools to negatively or positively modulate HSPB1(27) function

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Human HSP27 (HSPB1) is a molecular chaperone sensor which, through dynamic changes in its phosphorylation and oligomerization, allows cells to adapt to changes in their physiology and/or mount a protective response to injuries. In pathological conditions, the high level of HSPB1 expression can either be beneficial, such as in diseases characterized by cellular degenerations, or be malignant in cancer cells where it promotes tumourigenesis, metastasis and anti-cancer drug resistance. Structural changes allow HSPB1 to interact with specific client protein partners in order to modulate their folding/activity and/or half-life. Therefore, the search is open for therapeutic compounds aimed at either down- or upregulating HSPB1 activity. In this respect, we have previously described two peptide aptamers (PA11 and PA50) that specifically interact with HSPB1 small oligomers and decrease its anti-apoptotic and tumourigenic activities. A novel analysis of the different HSPB1-interacting aptamers that were isolated earlier revealed that one aptamer (PA23) has the intriguing ability to stimulate the protective activity of HSPB1. We show here that this aptamer abolishes the dominant negative effect induced by the R120G mutant of αB-crystallin (HSPB5) by disrupting its interaction with HSPB1. Hence, developing structure-based interfering strategies could lead to the discovery of HSPB1-based therapeutic drugs.

1. Introduction

We and others have reported that HSPB1 expression enhances cellular resistance to heat shock [1,2], oxidative stress [3], apoptosis inducers [4–6] and a pleiotropic number of conditions or compounds that are deleterious to cells, such as anti-cancer drugs [7]. Recent studies have shown that HSPB1 has multiple strategies to attenuate the damage induced by stress challenges. For example, in stress that alters protein conformation, such as heat shock, HSPB1 acts as an ATP-independent holdase chaperone that targets and traps misfolded polypeptides in a refolding competent state hence avoiding their aggregation [8,9]. In oxidative conditions, in addition to its participation in the elimination of oxidized proteins, HSPB1 also responds by inducing a protective pro-reducing state [10] that probably results from the modulation of several anti-oxidant enzymes, such as glucose 6-phosphate dehydrogenase [11]. The negative effect of HSPB1 against apoptotic cell death [4] is the result of its ability to modulate key steps of the apoptotic cascade through interaction with crucial regulators, such as cytochrome c [12,13], pro-caspase-3 [14,15], DAXX [16], Akt [17], Stat3 [18], eIF4E [19] and F-actin, an upstream modulator of apoptosis [13]. Expression of HSPB1 is upregulated in many tumour cells (particularly those from breast, colon, ovary, head and neck) and is usually associated with a poor clinical outcome [20,21]. Indeed, high levels of HSPB1 are tumourigenic [22,23] and allow cancer cells to evade apoptotic death, proliferate and become metastatic [24–27]. Interaction of HSPB1 with key protein targets may explain, at least, in part, the role played by this protein in tumour promotion.
For example, HSPB1 modulates the activation of metalloproteinase type 2 that digests components of the extracellular matrix surrounding tumour masses [28] and interacts with β-catenin, and consequently modulates cadherin–catenin cell adhesion proteins that regulate tumour cell invasion [29].

Towards understanding the pleiotropic functions of HSPB1, recent studies have reported the crucial role played by the dynamic and complex oligomerization/phosphorylation of this chaperone [30,31]. In this respect, a crucial role is played by mitogen-activated protein kinase–activated protein kinase 2,3 (MAPKAP kinase 2,3) that phosphorylates HSPB1 at three serine residues. Our recent analysis of HSPB1 oligomerization/phosphorylation in cells exposed to different apoptotic inducers revealed that HSPB1 has multiple possibilities of forming different-sized and phosphorylated oligomers, suggesting that it can choose the more appropriate strategy to counteract apoptotic challenges triggered by unrelated inducers [30]. Another important feature is its ability to interact with a large number of client polypeptides, such as those we recently described: pro-caspase 3, histone deacetylase 6, and signal transducer and activator of transcription 2 [15]. Hence, in a particular cell condition, specific changes in oligomerization/phosphorylation may allow HSPB1 to interact with the more appropriate polypeptides to allow cells to mount an adaptive response. Through modulation of the activity and/or half-life of the targeted polypeptides, HSPB1 could then indirectly protect against the apoptotic inducer staurosporine. HeLa cells were transiently transfected with either empty vector (pCi Neo) or vectors encoding PAc, PA23 or shRNAHSPB1 (RNAi). Forty-eight hours after transfection, cells were treated for different time periods with staurosporine. Cell survival was estimated by crystal violet staining. The survival percentage corresponded to the ratio of the relative absorbance of the different samples to that of untreated cells. Note the high sensitivity of HeLa cells devoid of HSPB1 (RNAi) and the protection mediated by PA23.

(c) As in (b), but Neo, WT and R120G cells were transiently transfected with vectors encoding PAc (black bar) or PA23 (grey bar), before being exposed to increasing doses of menadione. In (b,c), the histogram bars are representative of at least three separate experiments, standard deviations are presented (n = 3). Similar results were obtained by monitoring cell death using Trypan blue staining.

Figure 1. Peptide aptamer 23 (PA23) enhances cellular resistance against apoptotic and oxidative stress. (a) Schematic of the organization of inactivated bacterial thioredoxin-based PAs. Peptide sequences of PAc, PA11, PA50 and PA23 are presented. The putative PA23 phosphorylated site is in bold. Another approach that can alter HSPB1 ability to promote interaction with particular client polypeptides appears as having multiple cellular functions, a phenomenon illustrated by the multiple and apparently unrelated effects associated with HSPB1 over- or underexpression.

Because of the deleterious role played by HSPB1 in several cancer pathologies, conditions or drugs that can invalidate or disrupt HSPB1 deleterious pro-cancer activity are actively sought. In addition to depleting HSPB1 by antisense or RNA interference (RNAi) strategy [27,32,33], disturbing its structural organization and subsequently its interaction with particular client polypeptides appears as another approach that can alter HSPB1 ability to promote tumour growth. For example, the drug brivudine improves the efficiency of human pancreatic cancer chemotherapy by impairing the ability of HSPB1 to block apoptosis through the recognition of pathological protein substrates [34]. We have been interested in the peptide aptamers (PAs) technology to target HSPB1 structure/function. Indeed, recent studies have shown that PAs can successfully modulate the activity of a wide range of intracellular proteins involved in cell growth regulation, such as cyclin-dependent kinase 2 (CDK 2) [35], epidermal growth factor receptor [36], calcineurin [37], BCL-6 [38] and Nr-13 [39]. These studies illustrate the power of phenotypic screening of combinatorial peptide libraries in order to interrogate the proteome and chart molecular regulatory networks. Moreover, one advantage of PAs over RNAi is that their amino acid sequence can be used in the design of peptidomimetic drugs.

2. Peptide aptamers that counteract the protective activity of HSPB1

A few years ago, we performed an intensive search for PAs that specifically interact with HSPB1. This earlier study led to the discovery of two PAs (PA11 and PA50) that were highly efficient in negatively modulating HSPB1 protective activity [40]. The first step was to select PAs that specifically interact with HSPB1. This was carried out using an improved version of the yeast two-hybrid interaction trap, essentially as previously described [41]. In this system, prey were a mix of randomized 8-mer and 12-mer PA libraries in pWP2 vector [41]. Peptides of random sequence were inserted into a scaffold protein platform made of inactivated Escherichia coli thioredoxin A with a conformationally constrained sequence that stabilizes the conformation of the inserted peptide (figure 1a). The corresponding baits were either wild-type (WT) or mutants of HSPB1 fused into the LexA yeast plasmid. Another member of the small heat-shock protein
(sHSP) family, HSPB5 and its myopathy-inducing R120G mutant were used as bait controls. Interactions were detected by interaction mating assay between the MB210a (MATα) strain expressing the PA libraries and the MB226 (MAT) strain transformed with LexA–HSPB1 vector [41]. Interactions were confirmed after retransformation of bait and aptamer plasmids in EGY42 (MATα) and TB50 (MAT) yeast strains, respectively. Fifty PAs showing strong interaction phenotypes with LexA–HSPB1 were identified from an initial screen of $11 \times 10^6$ yeast colonies [40]. The I/LLRRL/I consensus sequence was identified in 50 per cent of them. Analysis of the interaction with various mutants revealed that these PAs recognized different regions of the HSPB1 molecule, outside of the C-terminal part of the crystallin domain (position 141–175). After elimination of PAs that recognized HSPB5, aptamers were selected, recloned in pcDNA mammalian expression vector and expressed in a human cell culture system [40]. In parallel, control aptamers (PAc) unable to recognize a protein target were tested. Most human cells, particularly those of cancerous origin, constitutively express high loads of HSPB1 [13,30]. We have used HeLa cells, derived from cervical cancer, because they have the advantage of being efficiently transfectable and constitutively express a high level (5 ng μg$^{-1}$ of total proteins) of HSPB1. In these cells, HSPB1 constitutive expression generates cell death resistance, as demonstrated by antisense- and RNAi-mediated depletion approaches [13,30]. Among the different aptamers that were tested, two of them (PA11 and PA50), displaying different variable sequences (QLSGWVGRCL and YLLRRLCC, respectively), were the most efficient ones to interfere with HSPB1 protective activity. These aptamers were then further characterized.

(a) Peptide aptamers that interfere with the anti-apoptotic property of HSPB1

Following transient transfection of HeLa cells, expression of PA11 and PA50 slightly decreased cell growth and had the intriguing ability to enhance staurosporine-mediated apoptotic death more efficiently than by depleting HSPB1 [40]. Indeed, following 6 h of exposure to 0.1–0.125 μM of staurosporine, PA11 and PA50 stimulated cell death by at least 30 per cent compared with control or PAc-expressing cells. A similar observation was made in response to oxidative stress and the anti-cancer drugs cisplatin and doxorubicin. No protective effects of PAs were detected in HSPB1-depleted cells, hence confirming that the increase in apoptosis induced by the PAs resulted from their specific interaction with HSPB1. The pro-apoptotic effect of PA11 and PA50 was confirmed by analysing the cleavage activation processing of procaspase 3 [40]. We also discovered that these PAs radiosensitized human head and, neck squamous carcinoma SQ20B cells [32,40]. PA11 and PA50 interacted with HSPB1 small oligomers and, consequently, they probably altered the recycling between small and large oligomers. This leads to the redistribution of a fraction of HSPB1 in large oligomers concomitantly with a decrease in serine 78 phosphorylation. PAs appear therefore to deeply alter HSPB1 structural organization.

(b) Peptide aptamers that down-modulate the tumourigenic activity of HSPB1

To test whether PAs could have an in vivo anti-HSPB1 activity, PA11- or PA50-expressing SQ20B cells were injected in one leg of female nude mice. To circumvent inter-animal variability and also because SQ20B cells are non-metastatic, SQ20B cells expressing PAc were injected in the other leg of each animal. Of interest, tumour growth was strongly reduced (up to 80%) in PA11- and PA50-expressing cells compared with cells expressing PAc [40]. Moreover, the reduced tumour growth was far more intense than that mediated by a short hairpin RNA (shRNA)-mediated depletion of HSPB1 suggesting that targeting HSPB1 with PAs is more efficient than downregulating its expression. Further analysis revealed that PA11 and PA50 expression altered tumour cell proliferation rather than inducing their spontaneous cell death, a phenomenon characterized by high levels of the CDK inhibitor p21-waf1 expression in PA11- and PA50-expressing tumours [40].

3. A peptide aptamer that upregulates the protective activity of HSPB1

A further in-depth analysis of our 50 different HSPB1 interacting aptamers revealed that one of them (PA23) had the surprising ability to stimulate the protective activity of HSPB1. This was not the first time that a PA was described to stimulate the activity of a targeted polypeptide because one had already been shown to stimulate calcineurin activity [37]. Nevertheless, this intriguing observation prompted us to further analyse PA23. These new data are presented below.

(a) Aptamer 23 stimulates the cellular resistance to staurosporine and oxidative stress

As seen in figure 1b, analysis of HeLa cells transiently expressing PA23 had an attenuated staurosporine-induced apoptotic cell death. In the same assay, expression of an shRNA-targeting HSPB1 mRNA or the earlier-described PA11 and PA50 decreased HSPB1 protective efficiency, whereas PAc had no effect [40]. Similarly, PA23 provided a cellular protection against the oxidative damages induced by menadione (figure 1c), a compound that generates intracellular reactive oxygen species via redox cycling, and hydrogen peroxide (not shown). Of interest, the sequence of PA23 (YLLRRLCC) differed from that of PA50 (YLLRLRCLC) by the presence of a phosphorylatable serine site (underlined bold) followed by an arginine residue. This motif, also present at the level of HSPB1 phosphoserine 78, may be recognized by MAPKAPK2,3 kinases [42]. Hence, it cannot be excluded that a putative phosphorylation of PA23 could be an additional parameter that modulates HSPB1 structure/function.

(b) Aptamer 23 attenuates the oxido-sensitivity induced by the R120G mutant of HSPB5 by disrupting HSPB1 interaction with this dominant negative mutant

We next tested whether PA23 could modulate the effects induced by HSPB5, a member of the family of sHSPs that forms mosaic oligomeric structures with HSPB1 [43]. HSPB5 expression has been described to induce a protective effect and to upregulate cellular oxidoresistance [44]. By contrast, the R120G mutation abolishes the protective activity of HSPB5 [45] and promotes its abnormal phosphorylation,
aggregation, intracellular location and interaction with other sHSPs and intermediate filaments [46]. In vivo, this mutation induces cataract pathologies as well as myopathies and cardiomyopathies [47]. As a consequence of its chaperone activity, HSPB1 can attenuate HSPB5 R120G mutant aggregation [48]; however, this effect is hampered by the dominant negative effect of the R120G mutation towards HSPB1. Based on these considerations, HeLa cells constitutively expressing either the WT cells or R120G mutant of HSPB5 (R120G cells) were obtained, as well as control cells transfected with empty vector (Neo cells). WT and R120G cells have the very interesting characteristic of expressing similar levels of exogenous HSPB5 (WT or mutant) which corresponded to that of endogenous HSPB1 (approx. 5 ng µg⁻¹ of total proteins; [49]). As expected, expression of HSPB5 enhanced HeLa cell resistance to oxidative stress. By contrast, the HSPB5 R120G mutant induced an oxido-sensitivity (figure 1c) as the consequence of its dominant negative effect towards HSPB1 structural organization (figure 3). In regards to these cells, one question that immediately arises is related to PA23. Could this stimulatory aptamer counteract the oxido-sensitivity mediated by the R120G mutant and alter HSPB1 structural organization in such a way that it is no longer under the dominant negative effect induced by mutant HSPB5? This prompted us to test the effects mediated by PA23 in HeLa cells expressing or not WT or mutant HSPB5. WT, R120G and Neo cells were therefore transiently transfected with vectors encoding either PAc or PA23, and the sensitivity of the transfected cells against menadione toxicity was tested. As seen in figure 1c, PA23 enhanced the survival following menadione treatment of control and HSPB5-expressing cells and surprisingly also of cells expressing mutant HSPB5. The effect was particularly intense in cells exposed for 4 h with 100 µM menadione. Similar observations were made in response to hydrogen peroxide challenges (not shown). In these conditions, the presence of PA23 strongly attenuated the cytotoxicity observed in cells expressing mutant HSPB5. WT HSPB5-expressing cells also showed increased survival compared with control cells and consequently were only slightly affected by menadione.

(c) Aptamer 23 and phosphorylation of HSPB1 and HSPB5

As a first approach towards understanding the protective mechanism mediated by PA23, we tested its effect towards HSPB1 and HSPB5 phosphorylation in control and oxidative conditions. Similar to HSPB1, HSPB5 is phosphorylated at three serine sites (19, 45 and 59) located in the N-terminal region of the protein [50]. As seen in figure 2a, in non-treated cells, expression of PA23 did not modify the basal level of HSPB1 and HSPB5 phosphorylation. By contrast, a decreased phosphorylation was detected when Neo, WT and R120G cells were exposed to menadione. However, the effect was serine site- and cell-dependent. In the three cell types, the most intense decrease in HSPB1 phosphorylation (36–61%) was at the level of serine 15. In WT and R120G cells, serines 78 and 82 phosphorylation were also altered but to a lesser extent than serine 15. By contrast, in Neo cells, phosphorylation of these sites was only weakly altered. Concerning HSPB5, the most intense effect was at the level of serine 45 (55–69%) and serine 19 (51–54%). Only a slight decrease was noticed at the level of serine 59 (approx. 10%). Hence, by decreasing the phosphorylation of the serine sites located close to the N-terminus of the polypeptides (figure 2b), PA23 may interfere with the structure of the N-terminal domain of HSPB1 and HSPB5.

(d) Aptamer 23 and native sizes of HSPB1 and HSPB5

In growing untreated HeLa cells, HSPB1 has a particular native profile characterized by two heterogeneous structures with sizes between 50–200 and 200–700 kDa [30]. In the presence of HSPB5, both proteins were recovered in a unique mosaic complex heterogeneous in size (500–800 kDa). The same phenomenon occurred when the R120G mutant of HSPB5 was expressed. However, in this case, the common native size shifted towards higher molecular masses (600–900 kDa; figure 3). Native immunoprecipitation studies confirmed that HSPB1 quantitatively interacted with all WT or mutant HSPB5 polypeptides present in these complexes [49]. We have analysed the effects mediated by PAs on the oligomerization profile of HSPB1 and mutant HSPB5. R120G cells were therefore transiently transfected with vectors encoding either PAc or PA23, both coupled to a haemagglutinin tag. This tagging had no effect on the stimulatory activity of PA23. Similar levels of expression of PAc and PA23 were observed that did not alter HSPB1 or mutant HSPB5 levels of expression (figure 3e). PAc expression did not alter the HSPB1–mutant HSPB5 complex and was recovered in the low molecular weight fractions of the column (figure 3b). By contrast, in the presence of PA23, the common oligomerization profile of HSPB1 and mutant HSPB5 was lost. HSPB1 was now mainly in the 29–500 kDa range, whereas mutant HSPB5 remained in the form of very large structures (greater than 669 kDa) that had the tendency to aggregate (not shown) [51]. PA23 was recovered together with HSPB1 in two size populations in the range of 29–200 kDa. These observations indicate that, through its interaction with HSPB1, PA23 disrupts the complex formed by mutant HSPB5 and HSPB1. This should abolish the dominant negative effect induced by this mutant (figure 3f). By contrast, PA23 did not alter the complex formed by HSPB1 and WT HSPB5 (not shown). In this regard, experiments aimed at comparing the salt resistance of the interactions between HSPB1 and WT or mutant HSPB5 clearly concluded with a more rigid and less dynamic interaction in the case of mutant HSPB5 [49, 46]. Consequently, the binding of PA23 to HSPB1 ended up in the disruption of the complex formed with the R120G mutant of HSPB5. To the contrary, the complex formed with WT HSPB5 remained intact. Analysis of Neo cells, devoid of HSPB5 expression, revealed that PA23 interacted with HSPB1 in the range 50–200 kDa (not shown). Regarding the stimulatory activity of PA23, future experiments will have to check whether this is due to improved HSPB1 putative interactions with anti-oxidant enzymes or client proteins that stimulate the degradation of oxidized polypeptides.

4. Conclusions

The need for therapeutic drugs that either up- or downregulate HSPB1 cellular protective activity is actively investigated. Indeed, depending on the type of pathology, HSPB1 cellular protective activity can either have a beneficial effect, such as in diseases characterized by pathological cellular
degenerations, or be detrimental if the protective activity occurs at the level of cancer cells that evade death and proliferate. A first approach to solve the problem raised by the pathological effect of HSPB1 in cancer has been to target its level of expression by RNAi strategies. These studies confirmed HSPB1 as a therapeutic target [52]. However, to design chemical drugs that either up- or down-modulate HSPB1 activity is a complex task that has to take into account the multiple and dynamic combinatorial structures formed by HSPB1 that regulate its interactions with client protein partners. Hence, disturbing or favouring HSPB1 interaction with particular client polypeptides by acting on its structural organization is a new approach that could modulate HSPB1 activity. Unfortunately, the three-dimensional structures of HSPB1 oligomers are still not yet deciphered, hence, strategies aimed at theoretically designing molecules that could interfere with pathological HSPB1 interaction with pathological client protein is not an easy task. In spite of these considerations, the newly described anti-cancer drug brivudine, as well as our studies dealing with PAs, support the idea that modulations of HSPB1 protein interactome is a first step towards strategies for rational drug design of HSPB1-negative or -positive regulators. The results already obtained using PA11 and PA50 in tumours suggest that peptidomimetic drugs derived from their amino acid sequence should be far more efficient than the approach based on the RNAi-mediated elimination of HSPB1. Indeed, cells appear more affected by the presence of an aptamer–HSPB1 complex that cannot interact with pro-cancerous targets than by HSPB1 depletion. Indeed, cells may have difficulties overcoming HSPB1 aptamer poisoning, whereas the absence of this protein may be rapidly rescued via a new cell strategy. Another important point presented here concerns PA23, a PA that has a positive action towards HSPB1 activity. This information suggests that it will be possible to create drugs that could stimulate HSPB1 protective function in pathologies where this protein already plays a beneficial role, such as asthma, myocardial infarction or emerging degenerative diseases. The ability of PA23 to counteract the dominant negative effect of mutant HSPB5 through disruption of the complex formed with HSPB1 is another example of the potential linked to this type of approach. This may lead to

Figure 2. Aptamer 23 decreases menadione-induced HSPB1 and HSPB5 phosphorylation. (a) Immunoblot analysis of total cellular proteins. Neo, WT and R120G cells, transiently transfected with either PAc (C) or PA23 (23) vector, were either kept untreated (NT) or treated for 2 h with 60 μM menadione (M). Total cellular protein extracts were analysed in immunoblots probed with antibodies specific for HSPB1 phosphorylation at the level of serines 15 (ser15), 78 (ser78) or 82 (ser82) and HSPB5 phosphorylation at the level of serines 19 (ser19), 45 (ser45) or 59 (ser59). Autoradiographs of revealed immunoblots are presented. (b) Quantitative analysis of the decreased phosphorylation induced by PA23 expression in menadione-treated cells. Percentage was calculated as the ratio between the level of phosphorylation of a defined serine site in PA23-expressing cells to that observed in cells expressing PAc.
the discovery of drugs that will rescue the function of sHSPs poisoned by their interaction with a mutated member of this family of proteins.

Appendix A. Methods for figures

(a) Figure 1: vectors, transfections and cell viability assay
DNAs encoding PAs were cloned in the mammalian expression vector pCiNeo (Promega). HSPB1-directed shRNA vector was produced using pSuperNeo vector (OligoEngine). Cloning was also undertaken in the Clonetech haematoglutinin-tagged pCMV–HA vector; the resulting vectors were denoted PAc–HA and PA23–HA. Transfection of Neo, WT or R120G cells or plain HeLa cells was performed using the lipofectamine procedure (Life Technologies). Forty-eight hours later, cells were seeded in 96-well plates (7.5 × 10^3 per well). Twelve hours later, they were treated for different time periods with several concentrations of staurosporine or menadione (Sigma). After treatment, the culture medium was discarded, and the remaining viable cells were rinsed twice in phosphate-buffered saline (PBS), and stained for 15 min with 0.5 per cent crystal violet in 50 per cent methanol. Afterwards, plates were rinsed and dried. Thereafter, the stained cells were solubilized using 0.1 M sodium citrate (pH 5.4) in 20 per cent methanol. The absorbance of each well was read at 570 nm with a Wallac 1420 multilabel counter (Perkin-Elmer). The percentage of cell survival corresponded to the ratio of the relative absorbance of the different samples to that of untreated cells. All experiments were in triplicate.

(b) Figures 2 and 3: immunoblots and gel filtration analysis
HeLa cells were washed in ice-cold PBS (pH 7.4), scraped from the dish and pelleted for 5 min at 1000g. They were then directly resuspended in SDS sample buffer and boiled. Samples were then subjected to SDS–PAGE and immunoblot analysis using appropriate antibodies. Harvested cells were also resuspended in a cold lysis buffer made of Tris–HCl, pH 7.4, 20 mM; NaCl, 20 mM; MgCl₂, 5 mM; EDTA, 0.1 mM and 0.1 per cent Triton X-100. The lysates were centrifuged at 10 000g for 10 min, and the supernatant was loaded on 6B gel filtration columns as described in appendix A. The presence of HSPB1, mutant HSPB5 and aptamer–HA in the fractions eluted from the column was detected in immunoblots probed with the corresponding antibody. Autoradiographs of revealed immunoblots are presented. Gel filtration markers (29, 66, 150, 200, 443, 669 kDa) are indicated. Exclusion size of the column is 2000 kDa. Note that HSPB1 and mutant HSPB5 have lost their ability to form a common mosaic complex in cells transiently expressing PA23. (d) Schematic of the disrupting effect of PA23 towards HSPB1–mutant HSPB5 polydispersed oligomeric complexes.

Figure 3. Disruption of HSPB1–R120G mutant HSPB5 interaction by aptamer 23. (a) To detect the expression of peptide aptamers (PAs), R120G cells were transiently transfected with either empty (Contr, no aptamer), PAc–HA (PAc) or PA23–HA (PA23) vector and total protein extracts were analysed in immunoblot probed with anti-HA, anti-HSPB1 and anti-HSPB5 antibodies. Autoradiographs of enhanced chemiluminescence (ECL)-revealed immunoblots are presented. Note that PAc–HA has a shorter migration than aptamer PA23–HA owing to the different length of the inserted peptide. (b,c) Analysis of HSPB1 and HSPB5 native size in R120G cells expressing either (b) PAc–HA or (c) PA23–HA vector. Forty-eight hours after transfection, cells were lysed and the 10 000g soluble fractions were applied to sepharose 6B gel filtration columns as described in appendix A. The presence of HSPB1, mutant HSPB5 and aptamer–HA in the fractions eluted from the column was detected in immunoblots probed with the corresponding antibody. Autoradiographs of revealed immunoblots are presented. Gel filtration markers (29, 66, 150, 200, 443, 669 kDa) are indicated. Exclusion size of the column is 2000 kDa. Note that HSPB1 and mutant HSPB5 have lost their ability to form a common mosaic complex in cells transiently expressing PA23. (d) Schematic of the disrupting effect of PA23 towards HSPB1–mutant HSPB5 polydispersed oligomeric complexes.

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