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PERSPECTIVE AND REFLECTION ARTICLE

Versatility of the small heat shock protein HSPB6 (Hsp20)

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Abstract The recently published review by Dreiza et al. (Cell Stress and Chaperones DOI 10.1007/s12192-0090127-8) dealing with the functional role of HSPB6 in muscle regulation is critically analyzed. Published data indicate that the chaperone-like activity of HSPB6 is comparable with that of HSPB5 and that phosphorylation of HSPB6 does not affect its oligomeric structure. Different hypotheses concerning the molecular mechanisms of HSPB6 action on smooth muscle contraction and on the reorganization of the cytoskeleton are compared, and it is concluded that although HSPB6 is not a genuine actin-binding protein, it can affect the actin cytoskeleton indirectly. Phosphorylated HSPB6 interacts with 14-3-3 and thereby displaces other binding partners of 14-3-3; among them, certain phosphatases, protein kinases, and various actin-binding proteins, which can participate in the reorganization of the actin cytoskeleton. In addition, HSPB6 seems to regulate the activity of certain protein kinases. All of these processes are dependent on HSPB6 phosphorylation which in turn might be regulated by the formation of heterooligomeric complexes of HSPB6 with other small heat shock proteins.

Keywords HSPB6 · Phosphorylation · Actin · 14-3-3 protein

We have read with great interest the recently published mini review by Dreiza et al. (2009). It contains important information on the role of small heat shock protein HSPB6 in the regulation of muscle contraction and the utilization of the short peptide derived from this protein for regulation of muscle tone and cytoskeleton. This review contains a compendium of data of interest to physiologists and medical doctors and combines the achievements of basic science with the demands of practical medicine. However, there are several issues that remain controversial or over-simplified and require brief comments:

1. Analyzing published data, the authors postulate that HSPB6 has lower chaperone-like activity than HSPB5. Indeed, low chaperone-like activity of HSPB6 was described by van de Klundert et al. working with recombinant rat HPSB6 that was isolated under rather harsh conditions (van de Klundert et al. 1998). However, later published investigations performed on untagged recombinant human HSPB6 purified under mild conditions indicated that the chaperone-like activity of HSPB6 is comparable or even higher than that of HSPB5 (Bukach et al. 2004).
2. Dreiza et al. postulate that phosphorylation leads to dissociation of the macromolecular aggregates of HSPB6 in the carotid artery (Dreiza et al. 2009). We suppose that it is necessary to refine the term "macromolecular aggregates." The size-exclusion chromatography of homogenate obtained from carotid artery before and after stimulation by forskolin or subjected to phosphorylation by cAMP-dependent protein kinase indicates that phosphorylation induces a decrease in the apparent molecular mass of the fractions containing HSPB6 (Brophy et al. 1999a, b). This means that phosphorylation somehow affects the composition or content of protein complexes containing HSPB6 but not that phosphorylation affects the quaternary structure of homooligomers of HSPB6 as it was interpreted in the literature and summarized by

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Salinthone et al. (2008). Indeed, experiments performed on phosphorylation mimicking mutant S16D of HSPB6 indicate that this mutation (and probably phosphorylation) does not affect the oligomeric structure of HSPB6 but decreases its chaperone-like activity (Bukach et al. 2004).

3. The authors pay special attention to the description of HSPB6-induced relaxation of smooth muscle. They present only one hypothesis and postulate that phosphorylated HSPB6 displaces phosphorylated cofilin from its complex with 14-3-3. This process is followed by cofilin dephosphorylation and actin depolymerization leading to smooth muscle relaxation. We suppose that it is necessary to present and discuss other hypotheses formulated in the literature. Brophy et al. postulated that HSPB6 is an actin-associated protein and that phosphorylated HSPB6 predominantly interacts with globular actin, whereas nonphosphorylated HSPB6 predominantly interacts with filamentous actin (Brophy et al. 1999a, b). At the same time, Rembold et al. came to the conclusion that phosphorylated HSPB6 binds to actin and, similarly to troponin I, inhibits cross-bridge cycling (Rembold et al. 2000). The mechanism of HSPB6 action proposed by the two abovementioned groups was completely different and was vividly discussed in many publications (reviewed by Gusev et al. 2005). However, the common viewpoint was that the smooth muscle relaxation induced by phosphorylated HSPB6 is due to its direct binding to actin (Flynn et al. 2003) or to certain actin-binding proteins (for instance, α-actinin; Tessier et al. 2003). It is worthwhile to mention that this hypothesis remains popular (Tyson et al. 2008; Hashimoto et al. 2009). We tried to check this hypothesis and analyzed the binding of HSPB6 to actin, reconstructed actin filaments, and myofibrils in vitro (Bukach et al. 2005). In all cases, the stoichiometry of binding was less than 0.04 mol of HSPB6 per mole of actin and was independent of phosphorylation or mutation (S16D) mimicking HSPB6 phosphorylation. This makes very questionable the direct involvement of HSPB6 in regulation of actin filaments. Moreover, in the smooth muscle, the intracellular concentration calculated per HSPB6 monomer (100–200 μM; Kato et al. 1994) is much lower than that of actin (1,000–1,100 μM; Rembold et al. 2000). In addition, HSPB6 is predominantly localized in the cytoplasm (Rembold and Zhang 2001; Woodrum et al. 2003) where it forms tight complexes with HSPB5 (Pipkin et al. 2003) and HSPB1 (Bukach et al. 2004, 2009). All of these facts make direct interaction of HSPB6 with actin very improbable. However, as mentioned earlier, the hypothesis on direct binding of HSPB6 to actin still remains popular in the literature.

4. According to Dreiza et al. (2005, 2009), phosphorylated HSPB6 (or its transducible phosphopeptide) induces relaxation of smooth muscle by displacing cofilin from its complex with 14-3-3. The liberated cofilin is dephosphorylated and induces smooth muscle relaxation by actin depolymerization. Although this is a very attractive hypothesis, many questions remain to be answered. Firstly, 14-3-3 recognizes and interacts with more than 200 different proteins (Pozuelo Rubio et al. 2004). Therefore, the question arises as to why phosphorylated HSPB6 specifically displaces only phosphorylated cofilin from its complex with 14-3-3. Published data (Dreiza et al. 2009) indicate that the displacement of cofilin was observed only at a rather high concentration of HSPB6 phosphopeptide. It is, therefore, desirable to compare the concentrations and apparent affinities of phosphorylated HSPB6 and phosphorylated cofilin (as well as other potential protein targets) to 14-3-3. It is known that 14-3-3 interacts with a number of proteins involved in regulation of actin dynamics (such as β-filamin, α-actinin, zyxin, calponin, etc.; Pozuelo Rubio et al. 2004), as well as with different protein kinases and protein phosphatases (Aitken et al. 2002; Pozuelo Rubio et al. 2004). For instance, 14-3-3 binds and inhibits slingshot phosphatase, which dephosphorylates and activates cofilin, and dephosphorylates and inactivates LIM-kinase 1, which in turn is involved in cofilin phosphorylation (Huang et al. 2006; Eiseler et al. 2009). We suggest that HSPB6-induced displacement of certain protein kinases (or protein phosphatases) from their complexes with 14-3-3 might be more effective in regulation of smooth muscle relaxation than displacement of phosphorylated cofilin (or any other proteins directly involved in regulation of actin dynamics). Secondly, published data (Bamburg 1999) indicate that approximately 30% of cofilin is phosphorylated and the portion of phosphorylated cofilin bound to 14-3-3 remains to our knowledge unknown. The effective regulation of smooth muscle relaxation only by HSPB6-induced displacement of phosphorylated cofilin from its complex with 14-3-3 is, therefore, questionable.

5. Analyzing the effect of HSPB6 on skeletal muscle the authors indicate that "HSPB6 has a binding domain to troponin 1 (Rembold et al. 2000), and it could be that HSPB6 can affect skeletal muscle contraction through troponin 1 and the troponin complex." The legend to Fig. 1 of Dreiza et al. paper (Dreiza et al. 2009) states "The troponin 1 binding motif is represented with a dotted line". Firstly, we assume that troponin 1 should be corrected to troponin I (Inhibitory subunit). Secondly, and more importantly, Rembold et al. postulated that

there is a region in the primary structure of HSPB6 that is similar to that of troponin I, but they did not suppose the presence of special troponin I binding domain in the structure of HSPB6 (Rembold et al. 2000). Thirdly, although the concentration of HSPB6 (calculated per monomer) in different muscle varies in the range of 5–200 μM (Kato et al. 1994), its concentration in fast skeletal muscle and heart is close to 10–20 μM and is less than that of troponin (30–70 μM; Robertson et al. 1981). Moreover, HSPB6 is predominantly located in cytoplasm (Pipkin et al 2003), whereas troponin is exclusively located on actin filaments (Potter 1974). All these facts make very improbable a direct interaction with and regulation of troponin by HSPB6.

The recently published reviews (Fan et al. 2005; Gusev et al. 2005; Dreiza et al. 2009) indicate that HSPB6 is involved in the regulation of many diverse processes such as relaxation of vascular muscle, myocardial contraction, myometrium functioning, platelet aggregation, and apoptosis. In addition, HSPB6 seems to participate in many pathological processes such as asthma, intimal hyperplasia, and insulin resistance. The question of why HSPB6 is so versatile remains unanswered. However, we hypothesize that the versatility of HSPB6 can be explained (at least partly) by its ability to interact with 14-3-3 (Chernik et al. 2007). Binding of phosphorylated HSPB6 to 14-3-3 can induce displacement of certain binding partners of 14-3-3 and among them pro- or antiapoptotic factors, protein kinases, protein phosphatases, and proteins involved in regulation of the actin cytoskeleton. The order and the nature of displaced proteins will be dependent on the abundance and affinity of the client proteins to 14-3-3 and on the availability of phosphorylated HSPB6. The unusual versatility of HSPB6 can also be explained by its direct involvement in the regulation of different protein kinases as was postulated by Fan et al. in recently published papers (Fan et al. 2006, 2008). Both these processes seem to be dependent on HSPB6 phosphorylation, which in turn might be regulated by formation of heterooligomeric complexes of HSPB6 with the other small heat shock proteins (Bukach et al. 2009).

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