Phosphorylated HSP27 Associates With the Activation-Dependent Cytoskeleton in Human Platelets

By Yan Zhu, Sarah O'Neill, Jeremy Saklatvala, Laura Tassi, and Michael E. Mendelsohn

The three prominently phosphorylated 29-kD proteins in thrombin-activated human platelets are forms of the mammalian 27-kD heat-shock protein (HSP27). Though the function of HSP27 is not yet known, its phosphorylation is highly correlated with platelet secretion, and recent evidence in nonhematopoietic cells suggests that HSP27 regulates cortical actin filament assembly. Therefore, the subcellular location and phosphorylation state of HSP27 in resting and thrombin-activated platelets was studied. Platelets were fractionated by established Triton X-100 lysis methods followed by differential centrifugation to obtain the 14,000g fraction (low-speed cytoskeleton), the 100,000g fraction (membrane skeleton), and the 100,000g supernatant fraction containing soluble cytosolic proteins. In resting platelets, HSP27 was present principally in the 100,000g supernatant fraction. Platelet activation with thrombin led to translocation of the majority of HSP27 to the low-speed cytoskeleton. This association was reversible by DNase, supporting the idea that HSP27 is a specific component of the actin cytoskeleton.

Activation of Platelets by agonists such as thrombin leads to a series of morphologic and biochemical changes, formation of platelet aggregates, secretion of platelet granules, and expression of surface-associated procoagulant activity. After agonist-induced activation, platelet cytoskeletal structures rapidly assemble. Though the mechanism and functional significance of many of these associations is not yet clear, proteins important to platelet participation in hemostasis (including GPIb-IX, GPIIb-IIIa, GPⅠla-Ⅱb, platelet factor XIII), cell-cell interactions (fibrogen, PECAM-1), and signaling (including Rap1b, protein kinase C, PI-3-kinase, focal adhesion kinase p125 FAK, and pp60 src) become quantitatively associated with the activation-dependent cytoskeleton.

Three acidic 29-kD proteins are rapidly and prominently phosphorylated after platelet activation by thrombin, and their phosphorylation is highly correlated with platelet dense granule secretion. We recently used antibody cloning methods to identify these 29-kD proteins as forms of HSP27, the unique low-molecular-weight (27 kD) heat-shock protein. Evidence suggests HSP27 participates in thermotolerance in mammalian cells, and the ability of HSP27 to function as a molecular chaperone has also been recently demonstrated. However, HSP27 is widely expressed in the absence of stress, and studies in many cells have now shown phosphorylation of HSP27 by physiologic agonists, suggesting a more general role for the protein in cellular signal transduction. In addition, HSP27 phosphorylation is not essential for the known chaperone or thermoresistance properties of the protein, suggesting this modification is important for some other function. HSP27 is phosphorylated rapidly after platelet activation by thrombin or receptor tyrosine kinases, and is also the major phosphosubstrate in fibroblasts activated by interleukin-1 (IL-1), tumor necrosis factor, or thrombin. HSP27 is similarly a prominent phosphoprotein in endothelial cell and smooth-muscle cell—signaling events.

Recent data strongly suggest HSP27 interacts with and regulates the actin cytoskeleton. Overexpression of HSP27 in fibroblasts increases stress fiber stability during hyperthermia, prevents cytochalasin D—mediated actin depolymerization, and leads to increased cortical filamentous actin, ruffling, and pinocytotic activity. Overexpression of nonphosphorylatable HSP27 inhibits each of these activities, yielding a phenotype identical to a dominant inhibitory mutant of the guanosine triphosphate binding protein, rac1. HSP27 is phosphorylated by MAPKAP2 kinase and/or a novel kinase, and is dephosphorylated principally by phosphatase PP2A. Taken together, these data suggest HSP27 lies on a signaling pathway downstream from MAP kinase that culminates in cortical actin assembly. However, the precise function of HSP27 and the role of HSP27 phosphorylation in cellular activation is not understood, despite numerous biochemical and genetic studies. In this report, we show HSP27 is translocated to the platelet cytoskeleton after thrombin activation, that it is principally the phosphorylated forms of the protein that become cytoskeletal, and that phosphorylation immediately precedes the association of HSP27 with the activation-dependent cytoskeleton.

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From the Molecular Cardiology Research Center, New England Medical Center, Tufts University School of Medicine, Boston, MA; and Cytokine Laboratory, Babraham Institute, Cambridge, UK.

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Address reprint requests to Michael E. Mendelsohn, MD, Molecular Cardiology Research Center, New England Medical Center, 750 Washington St, Box 80, Boston, MA 02111.

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MATERIALS AND METHODS

Platelet Isolation and Separation

Platelets were isolated from healthy volunteers by differential centrifugation and platelet-rich plasma was prepared and gel filtered as described. In brief, platelets were pelleted in the presence of 1 μmol/L prostaglandin E1 (PGE1; Sigma Chemical Co, St Louis, MO) and then washed once with and resuspended in buffer A (130 mmol/L NaCl/10 mmol/L trisodium citrate, 9 mmol/L NaHCO3, 6 mmol/L dextrose, 0.9 mmol/L MgCl2, 0.81 mmol/L KH2PO4, and 10 mmol/L TRIS-HCl, pH 7.3). Gel filtration was performed on Sepharose 2B (Pharmacia, Uppsala, Sweden) and equilibrated in buffer A, to which CaCl2 was added to a final concentration of 1 mmol/L, and platelet aliquots were subjected to the various experimental conditions. For phosphorylation experiments, platelets were resuspended at a concentration of 0.5 to 1 × 108 platelet/mL, in 2 mL of buffer A containing 1.0 mCi of [32P]orthophosphate (Amersham, Arlington Heights, IL) at 37°C for 1 hour, after which they were gel filtered and entered into experiments.

Isolation of Platelet Activation-Dependent Cytoskeleton

The methods of Fox were followed to fractionate Triton-X 100 lysates of washed human platelets. Platelets were used at concentrations of 0.2 to 1 × 109/mL, and care was taken to use equal quantities for control and thrombin-activated samples. Control platelets were stirred in buffer A for 2 to 5 minutes (see Results) and then lysed. To activate platelets, platelet suspensions in PGE1-free buffer A were treated with a final concentration of 1 mmol/L of 5% Triton X-100, 10 mmol/L EGTA, 2 mmol/L phenylmethylsulfonic fluoride (pMSF), 100 mmol/L benzamidine, 5 μg/mL leupeptin, and 100 mmol/L TRIS-HCl, pH 7.4) and the activation-dependent (low-speed) cytoskeletons were isolated by centrifugation at 4°C at 14,000 g for 10 minutes. The supernatant was next removed and centrifuged at 4°C at 100,000 g for 2 hours to obtain the 100,000 g pellet (membrane skeletal fraction) and 100,000 g supernatant. Fractions were either mixed with sodium dodecyl sulfate (SDS)-sample buffer and boiled for electrophoresis or resuspended in buffer C (50 mmol/L NaCl, 50 mmol/L NaF, 5 mmol/L EDTA, 20 mmol/L Na pyrophosphate, 1 mmol/L PMSF, 1 mmol/L orthovanadate, 1% Triton X-100, and 20 mmol/L TRIS-HCl, pH 7.4) for immunoprecipitation as described below.

Experiments using DNase I (Boehringer Mannheim, Mannheim, Germany) to solubilize actin filaments were performed as described by Fox et al. Lysates were incubated on ice for 2 to 4 hours in the presence of DNase or control buffer, separated by centrifugation at 14,000 g, and resolved and immunoblotted as above.

Electrophoresis and Immunoblotting

One-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the procedure of Laemmli et al. Two-dimensional (2D) immunoelectrophoresis (IEF)/SDS-PAGE was by the methods of Celis and Bravo as reported. For immunoblotting, proteins were transferred to nitrocellulose, blocked in 5% dry milk, incubated sequentially with anti-HSP27 antibody D5 (Amersham International, UK or kind gift of R.J.B. King, Imperial Cancer Research Fund, UK) and goat-antimouse IgG horseradish peroxidase conjugate (Amersham) and developed by 3,3'-diaminobenzidine (Sigma) or enhanced chemiluminescence (Amersham). For the time-course studies, platelets were incubated for various times after thrombin addition, lysed in buffer B, separated and resolved as described above. HSP27 was quantitated by densitometry (Model 300A, Molecular Dynamics, Sunnyvale, CA).

Immunoprecipitation From the Cytoskeleton Fractions

To study the location of the HSP27 phosphoforms, and to search for proteins associating with HSP27, a solubilization/immunoprecipitation method was developed to isolate HSP27 from the three fractions described above. The 14,000 g and 100,000 g pellets, as well as the 100,000 g supernatant, were resuspended in buffer C and sonicated with a tip sonicator for 10 to 15 bursts of 10-10 seconds each, so that all pellets were completely solubilized. To verify solubilization, after transfer of the solubilized fractions to fresh tubes, SDS buffer was added to the original tubes and heated to 100°C for 5 minutes, subjected to SDS-PAGE, electrotransferred, and immunoblotted with anti-HSP27 antibody. Resuspension of HSP27 from the pellets was complete, as judged by the absence of any immunoreactive HSP27 remaining in the original tubes on these immunoblots. Resuspended lysates were precleared with Protein A-Sepharose (Pharmacia) and immunoprecipitated as we described with murine monoclonal anti-HSP27 antibody D5. Control studies in parallel samples without the addition of D5 antibody were negative for any detectable HSP27 (see Results).

Immunofluorescence Microscopy

Resting platelets. Fresh platelets were prepared as described above and resuspended in buffer D: (10 mmol/L HEPES, 145 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl2, 10 mmol/L glucose, and 1 μmol/L of PGE1, pH 7.4). Resting platelets were fixed and studied by the methods of Hartwig and DeSisto with minor modifications. Resuspended platelets were incubated for 10 minutes at 37°C and fixed by addition of an equal volume of 4% formaldehyde in phosphate-buffered saline (PBS; pH 7.4) for 30 minutes. The fixed platelets were then gently pelleted, and resuspended in blocking solution containing 0.1 mmol/L lysine in PBS for 1 hour. After three washes in PBS containing 0.1% bovine serum albumin (BSA), the platelets were permeabilized with 0.5% Triton X-100 for 10 minutes, and washed three times. The platelets were then sequentially incubated with D5 antibody (1:20 dilution), fluorescein isothiocyanate (FITC)-antimouse IgG (1:200 dilution; Sigma), and rhodamine-phallodin (1:200 dilution; Sigma) for 1 hour each, with three buffer washes between each incubation. The platelets were finally resuspended in 10% PBS, 90% glycerol, 0.1% p-phenylenediamine (pH 8.0) and allowed to settle by gravity or gently centrifuged onto polylysine-coated coverslips, mounted, and examined. Control studies of platelets with FITC-antimouse IgG alone under identical conditions showed no stained cells.

Activated platelets. To study glass-activated platelets, cells were allowed to spread fully on glass coverslips, fixed with 3.7% formaldehyde, and permeabilized with 0.1% Triton X-100 in PBS, all exactly as reported. Cells were incubated with PBS + 0.5% BSA for 45 minutes, exposed to D5 antibody (1:5 dilution) for 60 minutes, washed with four exchanges of PBS-BSA, and then treated with 1:20 to 1:200 dilutions of FITC-conjugated antimouse IgG antibody for 60 minutes. After four additional washes in PBS-BSA, actin was stained with a 1:30 dilution of rhodamine-phallodin for 20 minutes. Cells were then washed four times in PBS-BSA, mounted, and photographed. Control stains of platelets with FITC-antimouse IgG alone under identical conditions were negative.

RESULTS

Association of HSP27 With the Activation-Dependent Cytoskeleton

To explore first the subcellular localization of HSP27 in resting and activated platelets, differential centrifugation
methods\textsuperscript{23} were used with Triton X-100 lysates of control (C) or thrombin-stimulated (THR) platelets to obtain the activation-dependent cytoskeleton (14,000g pellet), the membrane skeleton (100,000g pellet), and the solubilized membrane components/cytosolic proteins (100,000g supernatant). Total proteins were visualized by Coomassie blue staining (Fig 1A) and HSP27 was identified by immunoblotting (Fig 1B). As shown by previous investigators,\textsuperscript{5,6,10} the resting platelet low-speed cytoskeleton is principally comprised of actin (Fig 1A, 14,000g pellet, control). After activation, a number of proteins become localized to the low-speed pellet, including bands corresponding to the platelet structural proteins actin-binding protein (250 kD), myosin (210 kD), \(\alpha\)-actinin (100 kD), actin (43 kD), and myosin light chain (20 kD), as well as several proteins known to be central to platelet participation in hemostasis and thrombosis, including GpIb and the \(\alpha\), \(\beta\), and \(\gamma\) chains of fibrinogen (55 to 66 kD) (Fig 1A, 14,000g pellet, THR).\textsuperscript{5,6,10} The western blot in Fig 1B shows the majority of HSP27 in the resting platelet is found in the 100,000g supernatant, with a small amount of HSP27 detectable in the membrane skeleton fraction (100,000g pellet) and trace quantities of HSP27 detectable in the low-speed cytoskeletal pellet. With platelet activation by thrombin, the HSP27 protein becomes prominently associated with the activation-dependent cytoskeleton (Fig 1B, 14K pellet, THR). The band below HSP27 in the 14,000g pellet of thrombin-activated platelets was also present consistently, and likely represents a proteolytic fragment of HSP27 (see below).

To further show the association of HSP27 with the 14,000g pellet involves a specific cytoskeletal interaction, the ability of DNase I treatment to solubilize HSP27 from the pellet was examined. Figure 1C shows HSP27 present in the 14,000g cytoskeletal pellets after thrombin activation (lane 2) is effectively solubilized by 4 hours of DNase I treatment (lanes 3 and 6). Coomassie blue-stained gels from these experiments showed the expected parallel decrease in actin in the 14,000g pellet after DNase treatment (data not shown). Resolubilization of HSP27 from the cytoskeletal pellet also was directly correlated with the degree of depolymerization of actin at 2 (approximately 50%) and 20 hours (complete) of DNase I treatment (data not shown).

**Immunofluorescence Studies of HSP27 and Actin in Resting and Activated Platelets**

Because actin is the major filamentous component of the activated platelet cytoskeleton\textsuperscript{5} and HSP27 becomes a specific component of the low-speed cytoskeleton on platelet activation, immunofluorescence studies of actin and HSP27 were next conducted in both resting and glass-activated platelets. First, resting platelets were fixed and permeabilized in solution and then allowed to adhere to polylysine-coated coverslips (Fig 2). The staining pattern for HSP27 in resting platelets shows...
Fig 2. Immunofluorescence distribution of HSP27 and actin in resting platelets. Resting platelets were fixed in solution, allowed to adhere to polylysine-coated coverslips, and stained for HSP27 and actin. Two representative pairs of resting platelets are shown (original magnification x 100). (A) and (D), phase-contrast; (B) and (E), rhodamine-phalloidin staining for actin; (C) and (F), HSP27 staining. Note the punctate cytoplasmic aggregates of HSP27 (arrows). Bars represent 2.8 μm.

Platelets showed a cytoplasmic distribution, with a punctate pattern consistent with the HSP27 aggregates reported in other cells (Fig 2, C and F [arrows]). The actin pattern in resting platelets did not colocalize with HSP27, (Fig 2, B and E). Thus, the distinct staining patterns for actin and HSP27 in resting platelets were consistent with the biochemical data for control platelets in Fig 1.

The distributions of HSP27 and actin in fully spread, glass-activated platelets were studied next. Figure 3 shows the staining pattern of actin (A) and HSP27 (B) in activated platelets in the various stages of spreading. The images in Fig 3 show consistent colocalization of HSP27 and actin in the activated platelets (Fig 1, 14,000g pellet, THR). In separate studies, platelet cytoskeletons were isolated and prepared by treating glass-activated cells with 1% Triton X-100 before fixation and staining. These also contained for HSP27 and actin (data not shown), confirming the biochemical data for thrombin-activated platelets shown in Fig 1. The two fully spread cells in Fig 3 (arrows) suggest that HSP27 and actin colocalize in two specific regions: a peripheral, circumferential (subplasma membrane) region, and the dense central area where both cytoskeletal proteins and secretory granules are known to be concentrated on activation.

Association of Phosphorylated Forms of HSP27 With the Activation-Dependent Cytoskeleton

In resting platelets, though a low level of constitutive phosphorylation of HSP27 is observed, most HSP27 is unphosphorylated. During platelet activation by thrombin, the majority of the protein becomes phosphorylated to three increasingly acidic forms (29a,b,c). To determine the relative distribution of the phosphorylated forms of HSP27, resting and thrombin-activated platelets were labeled with 32P, fractionated as above into 14,000g and 100,000g pellets and 100,000g supernatant, and the fractions were then immunoprecipitated with anti-HSP27 antibody and studied in several ways (Fig 4). Silver-staining of immunoprecipitates from the platelet fractions is shown in Fig 4A. In addition to the heavy (55 kD) and light chain (27 kD) of the immunoprecipitating antibody, a small amount of cellular actin was seen to coprecipitate with HSP27 from the resting platelet cytoplasm (Fig 4A; 100,000g super, control, thin band at 42 kD). After thrombin activation, the majority of actin from the low-speed pellet was recovered in the immunoprecipitates (Fig 4A, 14,000g pellet, THR). A number of other proteins were detected in substoichiometric quantities in the immunoprecipitates from the resting platelet 100,000g supernatant and the 14,000g pellet after cell activation. However, the significance of these associations is not yet clear (see Discussion).

Immunoblotting was used next to quantitate the amount of HSP27 protein in each of the fractions from resting and activated 32P-labeled platelets. Immunoblotting of the immunoprecipitated fractions with anti-HSP27 antibody (Fig 4B)
showed HSP27 to be present principally in the 100,000g supernatant of resting platelets, and to be translocated to the low-speed pellet upon platelet activation (see also Fig 1B). The trace quantities of HSP27 detectable in the 100,000g pellet of resting cells in Fig 1 were not detected in these studies, perhaps because the epitope recognized by the anti-HSP27 antibody was not sufficiently available from this fraction.

Figure 4C shows an autoradiograph generated directly from the immunoblot in Fig 4B. In the resting platelet, only a small amount of constitutively phosphorylated HSP27 is detectable (Fig 4C, 100,000g super, control), consistent with our own and another study of HSP27 in resting platelets in which small amounts of constitutive phosphorylation of all three HSP27 phosphoforms 29a through c were reported. Indeed, 2D autoradiography of this 100,000g supernatant fraction showed small amounts of all three HSP27 phosphoforms (data not shown). The two larger phosphoproteins of approximately 200 kD and 110 kD seen in the 100,000g supernatant of resting platelets in Fig 4C were not specifically associated with HSP27, since they were also present in control studies from which antibody was omitted (data not shown). Several low molecular weight phosphoproteins with sizes ranging from 21 kD to 15 kD were also present in these immunoprecipitates from the resting platelet supernatants, but phosphoproteins of these sizes were not isolated from the HSP27 immunoprecipitates from activated platelets, raising the possibility these proteins associate with HSP27 in the cytoplasm of the resting cell. The identity of these proteins is unknown, though some may represent proteolytic fragments of larger phosphoproteins.

Figure 4C also shows clearly the expected marked change in HSP27 phosphorylation after thrombin stimulation. Of note, the vast majority of phosphorylated HSP27 was found consistently in the low-speed pellet, with less than 10% of the phospho-HSP27 remaining in the 100,000g supernatant (Fig 4C, 14,000g pellet, THR). Thus, the HSP27 protein localized to this low-speed, insoluble fraction was heavily phosphorylated, and represented essentially all the phospho-HSP27 present, whereas the HSP27 protein remaining in the 100,000g supernatant after thrombin activation was minimally phosphorylated (Figs 1B and 4B). When the autoradiograph in Fig 4C was developed for longer, a band just below HSP27 corresponding to the presumed ~25 kD proteolytic fragment of HSP27 seen in Fig 1B was also visible (data not shown).

To investigate which of the three known phosphoforms of HSP27 were translocated to the low-speed pellet of thrombin-activated platelets, 2D SDS-PAGE and autoradiography were performed on the immunoprecipitates from the 14,000g pellet of the activated platelets (Fig 5). These experiments showed all three phosphorylated forms of HSP27 (proteins 29a,b,c) to be present in the low-speed pellet. In addition,
Fig 5. Two-dimensional IEF/SDS-PAGE of the low-speed pellet from thrombin-activated platelets. The immunoprecipitate from the cytoskeletal (14,000 g pellet) fraction of thrombin-stimulated platelets (Fig 4) was resolved on 2D IEF/SDS-PAGE and subjected to autoradiography. All three platelet phosphoforms of HSP27 are present (29a,b,c). The myosin light-chain complex is identified by the open arrowhead. +, acidic; −, basic end of first dimension IEF gel.

the myosin light-chain complex (open arrowhead) and three unidentified phosphoproteins (two ~110 kD, and one ~70 kD) were also present, raising the possibility that these phosphoproteins associate with HSP27 in the activation-dependent cytoskeleton, either directly or through a shared interaction with actin or another protein.

Time Course of HSP27 Phosphorylation and Translocation to the Activation-Dependent Cytoskeleton

Time-course studies were also performed on platelets activated by thrombin, to determine the rate of HSP27 phosphorylation and the rate of HSP27 association with the low-speed cytoskeletal pellet (Fig 6). Phosphorylation of HSP27 occurred rapidly, peaking within 44 to 94 seconds, and remained maximally phosphorylated for at least 240 seconds (data not shown). These data are consistent with the time course of HSP27 phosphorylation quantitated by 2D autoradiography, which shows HSP27 phosphorylation reaches maximal levels by 60 seconds and remains fully phosphorylated for at least 4 minutes.21 Immunoblotting of the low-speed pellet fraction from thrombin-activated platelets at various time points shows translocation of HSP27 to this cytoskeletal fraction over a period of 120 seconds, though only 29% of HSP27 was present in this fraction by 60 seconds. As can be seen from Fig 6, whereas both phosphorylation and translocation appear to be complete within 2 minutes of thrombin activation, phosphorylation of HSP27 clearly precedes the association of HSP27 with the 14,000 g cytoskeletal fraction. Thus, the data suggest that cytoplasmic platelet HSP27 is first phosphorylated and then translocated from the cytoplasm to the assembling cytoskeleton.

DISCUSSION

Taken together, our results show that phosphorylated HSP27 is translocated to the assembling cytoskeleton during platelet activation and are consistent with the possibility that the phosphorylation of HSP27 that occurs with cell activation plays a role in the translocation and/or cytoskeletal interaction of the protein. The subcellular distribution of HSP27 after cell activation has been a matter of some controversy in the various organisms in which this question has been examined. Some studies have suggested HSP27 is localized to the nucleus in stimulated cells as in the report of HSP27 translocation from cytosol to nucleus after arsenite treatment in HeLa cells.67 However, past investigations focused primarily on heat-shocked cells or other stress conditions, and even under such conditions, nuclear translocation of HSP27 was not consistently observed. Collier and Schlesinger68 showed that HSP24, a HSP27 homolog in chicken embryo fibroblasts, aggregated to detergent-insoluble, perinuclear phase-dense granules after stress. In Drosophila, HSP27 also was found to associate with perinuclear structures that proved to be cytoskeletal.69 Rossi and Lindquist70 showed...
further that the intracellular localization of HSP26 in yeast is highly variable and raised the possibility that cellular metabolism and division may affect the locale and function of HSP27.

The platelet cytoskeleton is composed primarily of actin filaments. In unstimulated platelets, approximately 60% of actin is unpolymerized. During platelet activation there is a rapid burst in actin polymerization, and about 80% to 90% of actin is found as filamentous actin as a complex cytoskeletal ultrastructure is assembled. The association of proteins with the platelet activation-dependent cytoskeleton with a time course that parallels observed increases in actin content (filament formation) has been observed for several actin-binding proteins. Though proof of direct binding of HSP27 to actin in platelets and an understanding of the specific actin-binding mechanism of HSP27 remain to be studied, our data are consistent with a similar association between HSP27 and actin. HSP27 does not show any significant homology to known actin binding domains or to SH3 domains, a motif found in several cytoskeletal-associated proteins. Given the data presented here, specific binding of HSP27 to assembling actin filaments in platelets remains a plausible explanation for the translocation of HSP27 to the cytoskeleton with activation. The recent reports of Miron et al. that show an actin-binding protein from turkey gizzard highly homologous to HSP27, and of Lavoie et al. suggest that a role for HSP27 in stabilization of cortical actin filaments, ruffling, and pinocytosis, lend support to this model.

The immunoprecipitation experiments raise the possibility that several HSP27-associated proteins exist in platelets. In resting platelets, where HSP27 is mainly cytoplasmic, a small amount of actin is coprecipitated by anti-HSP27 antibody, as are several proteins identified by silver staining and phosphoproteins identified by autoradiography. Thrombin activation leads to a different pattern of proteins that appear to coprecipitate with HSP27. However, a direct association between any of these proteins and HSP27 remains to be established because these proteins may be associated with HSP27 indirectly through the actin cytoskeleton and/or non-specifically immunoprecipitated because of an association with actin.

Our data suggest that, like the larger heat-shock proteins, HSP27 may both function as a molecular chaperone during normal cellular events and interact directly with actin. HSP100 and HSP90 are both actin-binding proteins, and HSP90 may tether the glucocorticoid receptor to actin filaments and shuttle this steroid receptor from cytoplasm to nucleus. In addition, data suggest that HSP90 participates in the recovery of the intermediate filament network after heat stress. Thus, an association between HSP27 and the activation-dependent platelet cytoskeleton might serve several possible functions. HSP27 may bind to actin in order to chaperone other (associated) proteins to specific cellular locations and/or for specific cellular functions. We have recently found HSP27 specifically binds the transglutaminase factor XIII, in human platelets, and also colocalizes with this protein to the activation-dependent cytoskeleton. Alternatively, HSP27 may itself have unidentified functional properties that are important to the assembly of the actin cytoskeleton or to activation events occurring in cytoskeletal regions to which HSP27 is localized.

Whereas our data suggest that HSP27 phosphorylation is correlated to its cytoskeletal translocation during platelet activation, the specific role of HSP27 phosphorylation with activation is not yet clear. Conformational changes of HSP27 by phosphorylation are likely and could allow or promote an increased interaction between HSP27 and actin, or between HSP27 and other actin-associated proteins. Alternatively, phosphorylation could allow HSP27 to dissociate from the recognized aggregates it forms in the resting cell, facilitating the translocation of the protein to the cytoskeleton, though recent studies in nonhematopoietic cells on the effects of phosphorylation on HSP27 oligomerization have been controversial. Finally, phosphorylation may affect an unrecognized functional property of the protein that promotes its association with cytoskeletal elements. An understanding of the role of phosphorylation in the translocation of HSP27 to the cytoskeleton in platelets will likely require preparation of unphosphorylated and phosphorylated forms of the protein. Such studies in turn may provide insight into the mechanism for the recognized correlation between HSP27 phosphorylation and platelet secretory events.

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