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The 29-kDa proteins phosphorylated in thrombin-activated human platelets are forms of the estrogen receptor-related 27-kDa heat shock protein

(signal transduction/thrombosis/platelet activation)

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ABSTRACT **Thrombin plays a critical role in platelet activation, hemostasis, and thrombosis. Cellular activation by thrombin leads to the phosphorylation of multiple proteins, most of which are unidentified. We have characterized several 29-kDa proteins that are rapidly phosphorylated following exposure of intact human platelets to thrombin. A murine monoclonal antibody raised to an unidentified estrogen receptor-related 29-kDa protein selectively recognized these proteins as well as a more basic, unphosphorylated 27-kDa protein. Cellular activation by thrombin led to a marked shift in the proportion of protein from the 27-kDa unphosphorylated form to the 29-kDa phosphoprotein species. Using this antibody, we isolated and sequenced a human cDNA clone encoding a protein that was identical to the mammalian 27-kDa heat shock protein (HSP27), a protein of uncertain function that is known to be phosphorylated to several forms and to be transcriptionally induced by estrogen. The 29-kDa proteins were confirmed to be phosphorylated forms of HSP27 by immunoprecipitation studies. Thus, the "estrogen receptor-related protein" is HSP27, and the three major 29-kDa proteins phosphorylated in thrombin-activated platelets are forms of HSP27. These data suggest a role for HSP27 in the signal transduction events of platelet activation.**

Thrombin is the most potent physiologic agonist of platelet activation (1–3) and initiates platelet secretion and aggregation in the developing thrombus by activating several important signal transduction pathways (for review, see ref. 4). One thrombin receptor has recently been cloned and shown to have a novel proteolytic mechanism of activation (5). However, the subsequent steps in platelet activation by thrombin and the contributions of known signal transduction pathways to specific platelet activation events are less well understood.

Protein kinase-mediated events are ubiquitous in cellular activation (for reviews, see refs. 6 and 7). The kinases involved in cellular signaling are only partially characterized, however (8), and little is known about the specific cellular substrates phosphorylated in response to physiologic agonists. Platelet activation by thrombin leads to marked increases in the level of [^{32}P]phosphate incorporation by a number of proteins (2, 9, 10). Studies to date have emphasized the phosphorylation of a 20-kDa protein identified subsequently as the light chain of myosin and the 40- to 47-kDa protein kinase C substrate(s) (2, 4). Three unidentified 29-kDa proteins have also been shown to be phosphorylated in platelets activated by a variety of physiologic agonists (11) and to be similar or identical to proteins in fibroblasts (11) and endothelial cells (12).

The heat shock proteins consist of a number of highly conserved proteins thought to play a protective role in cells subjected to high temperature or other stresses (reviewed in refs. 13 and 14). Evidence suggests that these proteins are synthesized for reasons in addition to thermotolerance and are present (and often essential) for normal cell functions (14). The high molecular weight heat shock proteins, HSP90, HSP70, and HSP60 (GroEL), have been studied in greatest detail (14). These proteins have been implicated as molecular "chaperones" in some interactions, such as that between HSP90 and steroid receptor complexes (15) or between HSP90 and protooncogene products with tyrosine kinase activity (13). However, even with these two examples the precise function of the HSP90, the nature and role of other associated proteins, and the role of HSP90 phosphorylation remain unclear (13–15).

The low molecular weight heat shock proteins are the least well understood members of the heat shock protein family (13). At least three potential levels of regulation exist for the low molecular weight heat shock proteins: their synthesis is temperature-induced; they are induced as well at specific developmental stages at normal temperature; and they are phosphorylated in response to a wide variety of stimuli (13, 14). In this report we characterize three major 29-kDa proteins phosphorylated following platelet activation by thrombin, identify these phosphoproteins as forms of HSP27, and establish that the previously unidentified estrogen receptor-related protein (16) is HSP27.

MATERIALS AND METHODS

Reagents. Sepharose 2B, Tween 20, Kodak X-Omat film, and 3,3'-diaminobenzidine were purchased from Sigma. [^{32}P]Orthophosphate was purchased from Amersham. Nitrocellulose (0.45 μm) was purchased from Schleicher & Schuell. Poly(vinylidene difluoride) (PVDF) Immobilon membranes were from Millipore. Goat anti-mouse IgG horseradish peroxidase conjugate was purchased from Amersham. Protein A-Sepharose was from Pharmacia. D5 antibody, a murine monoclonal antibody against an unidentified 27- to 29-kDa estrogen receptor-related protein (16), has recently become available from Amersham International (U.K.). DNA sequencing gel solution, Hydrolink, was purchased from AT Biochem (Malvern, PA). Restriction endonucleases, DNA-modifying enzymes, and T3 and T7 promoter primers were from New England Biolabs. Electrophoresis supplies were from Bio-Rad. Human α-thrombin was from

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Abbreviations: IEF, isoelectric focusing; PVDF, poly(vinylidene difluoride).

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Enzyme Research Laboratories (South Bend, IN). All other reagents were the highest quality available commercially.

Platelet Isolation and Separation. Platelets were isolated from normal volunteers and gel-filtered platelets were prepared (17). All preparations and subsequent experiments were performed at 22°C. Platelets were pelleted and resuspended in buffer A (130 mM NaCl/10 mM trisodium citrate/10 mM Tris base/9 mM $NaHCO_3$/6 mM dextrose/0.9 mM $MgCl_2$/0.81 mM KH_2PO_4, pH 7.3). After filtration on Sepharose 2B, platelet aliquots were subjected to various experimental conditions and pelleted at appropriate time points. For phosphorylation experiments, platelets were resuspended for 1 hr in 2 ml of buffer A containing 1.0 mCi (37 MBq) of $^{32}P_i$, after which they were gel-filtered and entered into experiments as described (18). The term phosphorylation as used in this report is synonymous with the net increase in protein-associated ^{32}P as detected by autoradiography.

Two-Dimensional Gel Electrophoresis. Platelet pellets from 0.8–1.9 × 10^8 platelets (see figure legends) were resuspended in lysis buffer and subjected to two-dimensional isoelectric focusing (IEF)–SDS/PAGE essentially as described (19). Upon completion, gels were either stained with Coomassie brilliant blue and dried for autoradiography or removed directly for immunoblotting. Silver staining was performed by the method of Morissey (20). Molecular weight standards were used to provide reference values and to localize the 29-kDa proteins in all gels.

Immunoblotting. For immunoblotting studies, two-dimensional gels from ^{32}P-labeled platelets were electroblotted to PVDF membranes for 60–90 min at 500 mA, rinsed with phosphate-buffered saline (PBS), and blocked overnight (21). Blots were subsequently incubated with D5 antibody at a 1:400 dilution in PBS containing 0.05% Tween 20. Blots were rinsed extensively and developed using a horseradish peroxidase system followed by diaminobenzidine (22). Immunoblots from immunoprecipitation experiments were prepared from SDS/12.5% polyacrylamide minigels, and exposed to film or probed with a 1:1000 dilution of a murine monoclonal anti-HSP27 antibody (a gift from William McGuire, University of Texas, San Antonio), and developed as above. In some experiments, 2-mercaptoethanol was omitted from the SDS/PAGE sample buffer to prevent reduction of the D5 antibody itself.

Immunoprecipitation. Experiments were performed essentially as described (23) using a radioimmunoprecipitation assay (RIPA) lysis buffer (20 mM Tris, pH 7.4/50 mM NaCl/50 mM NaF/5 mM EDTA/20 mM sodium pyrophosphate/1 mM phenylmethylsulfonyl fluoride/1 mM Na_3VO_4/1% (vol/vol) Triton X-100. Immunoprecipitates containing 1–2 × 10^8 platelets were precleared with protein A-Sepharose prior to addition of D5 antibody (10–20 μl). After overnight incubation at 4°C with rocking, immune complexes were isolated by addition of protein A-Sepharose, pelleted, and washed three times at 4°C with RIPA buffer. The pellets were solubilized by addition of SDS/PAGE sample buffer and immunoblots were prepared as above.

Autoradiography. Dried gels or ^{32}P-labeled electroblots were exposed to X-Omat AR film at −70°C in cassettes equipped with DuPont Cronex intensifying screens for various times. Densitometry was performed on a Molecular Dynamics 300A computing densitometer (Sunnyvale, CA) using the integrated volume mode. Values were expressed in arbitrary densitometric units (ADU).

Immunoscreening of cDNA Libraries. Random- and oligo(dT)-primed human umbilical vein endothelial cell cDNA libraries were constructed in λgt11 as reported (24) and were gifts of S. Orkin (Children's Hospital, Boston). The platelet cDNA library in λgt11 was kindly provided by K. Clemetson (25). Immunoscreening of recombinant phage with monoclonal antibody D5 was essentially as described (26). Positive clones were plaque-purified by three additional rescreening steps to obtain a single plaque.

Isolation and Sequencing of Recombinant Phage DNA. Phage DNA was purified from plate lysates (27). Purified DNA was digested with *Eco*RI, insert fragments were isolated by electrophoresis in low-melting-temperature agarose gels, and the DNA inserts were then subcloned into *Eco*RI-digested plasmid vector pBluescript (Stratagene). The DNA insert was sequenced as a double-stranded form by the chain-termination method (28) with the enzyme Sequenase (United States Biochemical). Sequence analysis was performed using the EUGENE Software program developed at Baylor College and available through the Molecular Biology Computer Research Resource (Dana–Farber Cancer Institute, Boston).

RESULTS

Two-Dimensional IEF–SDS/PAGE Analysis of Protein Phosphorylation in Human Platelets. An autoradiograph of a two-dimensional gel from ^{32}P-labeled platelets following thrombin stimulation is displayed in Fig. 1. Increases in phosphorylation of a number of proteins were consistently observed in thrombin-stimulated platelets, including three major 29-kDa proteins (29a–c). Isoelectric points for these proteins (average of 10 gels) were as follows: 29a, 6.8; 29b, 6.5; 29c, 5.7. These three phosphoproteins form the subject of this report. Proteins 29a–c were not visible following staining of two-dimensional gels with Coomassie brilliant blue but could be clearly seen after silver staining (Fig. 2*A*). The relative amounts of the three proteins, determined by silver staining, were 29b >> 29a > 29c. The corresponding pattern of phosphorylation (Fig. 2*B*) demonstrated 29c to be most prominently phosphorylated, suggesting that the stoichiometry of phosphorylation of 29c is higher than that of 29a or 29b.

A small degree of phosphorylation of several cellular proteins is known to occur in resting platelets (9, 10, 29).

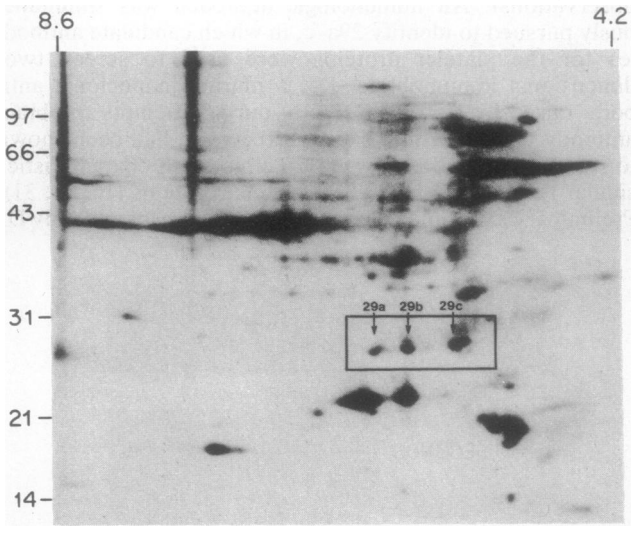

FIG. 1. Identification of 29-kDa phosphoproteins from ^{32}P-labeled human platelets. Lysate from 10^8 thrombin-stimulated (1 unit/ml, 2 min), ^{32}P-labeled platelets was prepared and subjected to two-dimensional IEF–SDS/12.5% PAGE on 12.5% gels. Shown is a representative autoradiograph from thrombin-stimulated platelets prepared in this manner and electroblotted to PVDF (39-hr exposure). Reference pH values for the IEF (first) dimension and molecular size standards (kDa) for the SDS/PAGE (second) dimension are marked and identify three principal, acidic 29-kDa proteins. These phosphoproteins, labeled 29a–c, are highlighted in the boxed area, which is detailed in Figs. 2–4.

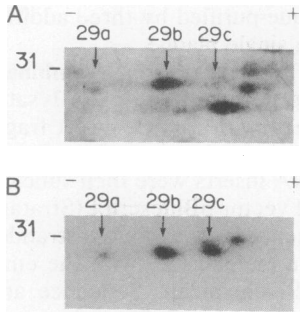

FIG. 2. Silver stain identification of the 29-kDa phosphoproteins in human platelets. A two-dimensional gel of 10^8 thrombin-stimulated (1 unit/ml, 2 min), ^{32}P-labeled platelets was prepared and subjected to silver staining (*A*) followed by autoradiography (*B*). The orientation of the acidic (+) and basic (−) ends of the first-dimension gel and the position of the 31-kDa molecular size marker are shown. The phosphorylated proteins 29a–c in *B* migrated exactly as in Fig. 1 and were directly superimposable on the silver-stained proteins bearing the same labels in *A*.

Phosphorylation of proteins 29a–c was minimal in resting platelets, with prominent increases in phosphorylation within 60 sec of stimulation by thrombin (Fig. 3). Densitometric analysis revealed that ^{32}P incorporation into proteins 29a–c increased 1.6-, 1.5-, and 3.3-fold, respectively, at 30 sec and 2.1-, 4.6-, and 7.1-fold, respectively, at 60 sec. The degree of phosphorylation at 60 sec was essentially the same as that at 2 min (*cf.* Fig. 4) or 4 min of thrombin exposure (unpublished observations). We consistently observed the small phosphoprotein adjacent to 29c as well, which may be a fourth protein form or an artifact (see below).

Immunoblot of ^{32}P-Labeled Platelet Proteins with D5, a Monoclonal Antibody Against an Unidentified Estrogen Receptor-Related 29-kDa Phosphoprotein. Extensive attempts to obtain protein sequence for 29a–c from two-dimensional gels transferred to PVDF were unsuccessful, in part because the amino termini of proteins 29a–c were blocked (unpublished observations). An immunologic approach was simultaneously pursued to identify 29a–c, in which candidate antibodies for the platelet proteins were used to screen two-dimensional immunoblots. D5, a murine monoclonal antibody raised by Coffer *et al.* (16) during attempts to obtain antibody to the purified estrogen receptor, had been shown to recognize 29-kDa phosphoprotein(s) with characteristics similar to those of the 29-kDa platelet proteins (16, 30, 31). Preliminary experiments confirmed the ability of antibody D5

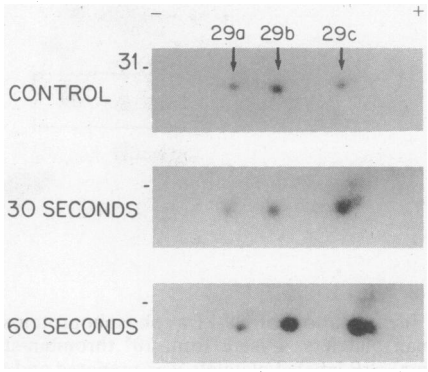

FIG. 3. Time course of phosphorylation of proteins 29a–c. ^{32}P-labeled platelets were analyzed by two-dimensional IEF–SDS/PAGE without stimulation (control) or following activation by human thrombin (1 unit/ml) for 30 or 60 sec. Care was taken to apply identical quantities of platelet lysate (1.1×10^8 platelets per gel) to each gel. Autoradiographs (9-hr exposure) of portions of the gels containing the 29-kDa proteins from these three time points are displayed.

to recognize 29-kDa proteins in samples from human platelets. Because of recent studies of the thrombin-induced phosphorylation of similar 28- to 30-kDa proteins in endothelial cells (12, 32, 33), we also examined first-passage ^{32}P-labeled human umbilical vein endothelial cells (kindly provided by M. Gimbrone, Brigham and Women's Hospital, Boston) and found that 29-kDa phosphoproteins were similarly detectable with the D5 antibody in these cells (unpublished observations).

Two-dimensional immunoblots from ^{32}P-labeled platelets demonstrated precise recognition of the phosphorylated proteins 29a–c by antibody D5 (Fig. 4 *Upper*). In addition, a more basic, unphosphorylated 27-kDa protein (arrowhead) was clearly recognized by D5 on the immunoblots. Strikingly, the predominant species in control (unstimulated) platelets was the unphosphorylated 27-kDa protein (Fig. 4 *Upper*), while stimulation of the platelets with thrombin led to a marked decrease in the amount of 27-kDa protein detected and an increase in the amount of each of the three proteins 29a–c on the immunoblots. The phosphorylation pattern generated from these immunoblots demonstrated a parallel increase in the phosphorylated species 29a–c following thrombin stimulation (Fig. 4 *Lower*; *cf.* Figs. 1–3). Densitometry revealed increases in phosphorylation at 2 min of 2.2-, 5.3-, and 7.7-fold for proteins 29a–c, respectively. These experiments established that the increase in the intensity of phosphoproteins 29a–c by autoradiography was due to an increase in the quantity of proteins 29a–c, as demonstrated by immunoblotting. The small species adjacent (acidic) to 29c was also demonstrable on both the immunoblot and the autoradiograph, suggesting that it, too, was either a separate form of the 27-kDa protein or artifactual and derived from protein 29c (*cf.* Figs. 1–3).

The data in Fig. 4 provided strong evidence that the 29-kDa phosphoproteins were all forms of the same protein, the unphosphorylated 27-kDa species present on the immunoblots. This was further supported by previous work in platelets and fibroblasts demonstrating identical electrophoretic patterns for these proteins following limited proteolysis with *Staphylococcus aureus* V8 protease (11). The identity of

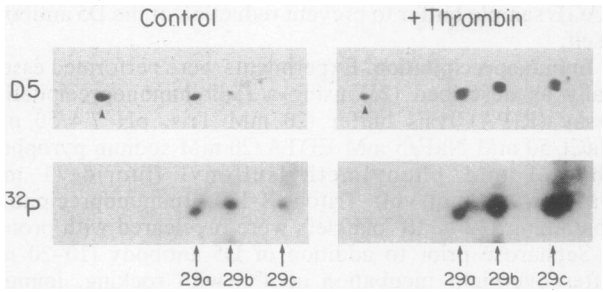

FIG. 4. Recognition of the 29-kDa phosphoproteins by D5, a monoclonal antibody directed against an uncharacterized estrogen receptor-related 29-kDa protein. Two-dimensional gels of ^{32}P-labeled platelet lysates (10^8 platelets per gel) were prepared from unstimulated platelets (control) or platelets exposed to human thrombin for 2 min (thrombin). Proteins were then electroblotted to PVDF and probed with D5, an antibody raised against a 29-kDa phosphoprotein copurified with the estrogen receptor (16). The immunoblots (*Upper*) were developed and visualized, dried, and placed on film to generate the corresponding autoradiographs (*Lower*). Only a portion of the two-dimensional immunoblots and autoradiographs is displayed (*cf.* Fig. 1). The phosphoproteins 29a–c (*Lower*) were localized by pI and molecular size as in Fig. 1, and were superimposable on the corresponding proteins of the immunoblots displayed (*Upper*). Along with proteins 29a–c, a more basic 27-kDa protein was evident on the immunoblots (arrowheads). No other proteins were detected anywhere on the two-dimensional immunoblots. The control immunoblot contained a faint protein corresponding to 29c that is not well reproduced in the figure.

the protein recognized by the D5 antibody to the estrogen receptor-related protein was determined next using molecular cloning techniques.

Screening of Human λgt11 Expression Libraries with Monoclonal Antibody D5, Isolation of cDNA, and Identification of the Clone Recognized as Expressing the 27-kDa Heat Shock Protein. Human λgt11 cDNA expression libraries from platelets (25) and endothelial cells (24) were screened. Screening of ≈150,000 plaques each from a human platelet cDNA library and an oligo(dT)-primed endothelial cell library led to the identification of a single plaque from the endothelial library recognized by the D5 antibody. The recombinant was plaque-purified and its DNA insert was subcloned into pBluescript for amplification and sequencing. Three hundred forty-eight bases were sequenced and the deduced amino acid sequence (Fig. 5) was used to search the Protein Identification Resource data base (National Biomedical Research Foundation; May 1991). The clone identified by the D5 antibody was identical to the mammalian 27-kDa heat shock protein, HSP27 (34).

To further confirm these findings, the D5 antibody was used in immunoprecipitation experiments with resting and thrombin-stimulated platelets (Fig. 6). First, immunoprecipitation studies using ^{32}P-labeled platelets were performed with the D5 antibody to demonstrate the resting phosphorylation and predicted increase in phosphorylated forms of HSP27 following thrombin stimulation. Immunoprecipitates were resolved by SDS/12.5% PAGE and subjected to autoradiography. A 3.2-fold increase in total 29-kDa protein phosphorylation was observed 2 min after stimulation with thrombin at 1 unit/ml (Fig. 6 *Left*). No other phosphorylated proteins were immunoprecipitated. In preliminary immunoprecipitation studies of the time course of phosphorylation, we found near-complete phosphorylation of the 29-kDa proteins within 10 sec following thrombin stimulation (unpublished observations), consistent with a role for these phosphorylation events in platelet signal transduction (2, 4, 10, 29). Further studies will be necessary, however, to quantitate precisely the time course for total 29-kDa phosphorylation and the individual time courses of phosphorylation for 29a–c and to correlate these with specific platelet activation events.

Next, a separate anti-HSP27 monoclonal antibody was used to probe immunoblots prepared from D5 immunoprecipitates of unlabeled platelets at rest or following thrombin stimulation (Fig. 6 *Right*). Immunoprecipitates were resolved in 12.5% gels, with 2-mercaptoethanol omitted from the sample buffer to prevent reduction of the D5 antibody. This omission had no effect on the migration of the immunoprecipitated 27- to 29-kDa proteins (unpublished observations). The proteins immunoprecipitated by the D5 antibody to the

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1                   21                  41
GGCAAGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGCGGAAA
GlyLysHisGluGluArgGlnAspGluHisGlyTyrIleSerArgCysPheThrArgLys

61                  81                  101
TACACGCTGCCCCCCCGGTGTGGACCCCACCCAAGTTTCCTCCTCCCTGTCCCCTGAGGGC
TyrThrLeuProProGlyValAspProThrGlnValSerSerSerLeuSerProGluGly

121                 141                 161
ACACTGACCGTGGAGGCCCCCATGCCCAAGCTAGCCACGCAGTCCAACGAGATCACCATC
ThrLeuThrValGluAlaProMetProLysLeuAlaThrGlnSerAsnGluIleThrIle

181                 201                 221
CCAGTCACCTTCGAGTCGCGGGCCCAGCTTGGGGGCAGAAGCTGCAAAATCCGATGAGAC
ProValThrPheGluSerArgAlaGlnLeuGlyGlyArgSerCysLysIleArg***

241                 261                 281
TGCCGCCAAGTAAAGCCTTAGCCCGGATGCCCACCCCTGCTGCCGCCACTGGCTGTGCCT

301                 321                 341
CCCCCGCCACCTGTGTGTTCTTTTGATACATTTATCTTCTGTTTTTCTC
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FIG. 5. Nucleotide and deduced amino acid sequence of the clone recognized by the D5 antibody. The 348 base pairs correspond to the 3′ end of the HSP27 gene, and the deduced 78-amino acid sequence is identical to the carboxyl terminus of mammalian HSP27 (34).

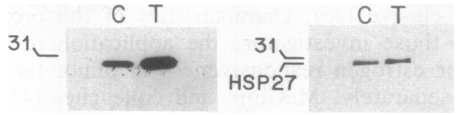

FIG. 6. Proteins immunoprecipitated from human platelets by the D5 antibody to the estrogen receptor-related protein are phosphorylated and are recognized by a distinct monoclonal antibody to HSP27. (*Left*) Platelet immunoprecipitates were prepared from ^{32}P-labeled control (lane C) and thrombin-activated (lane T) platelets with the D5 antibody and subjected to SDS/PAGE followed by autoradiography. (*Right*) In separate experiments, platelet lysates were immunoprecipitated using D5 antibody, subjected to SDS/PAGE, and probed with a distinct murine monoclonal antibody to HSP27. The proteins recognized by the anti-HSP27 antibody are indicated. The portion of each gel between 22 and 35 kDa is shown.

estrogen receptor-related protein were precisely recognized by a distinct monoclonal antibody to mammalian HSP27 (Fig. 6 *Right*). Except for the D5 antibody itself, no other proteins were recognized on these immunoblots.

DISCUSSION

Platelet activation in hemostasis and thrombosis is characterized by a number of protein phosphorylation events that are presumed to participate in the morphologic and secretory events that ensue. The present study characterizes the rapid incorporation of ^{32}P into three 29-kDa platelet proteins following cell activation by thrombin. The observed time course of phosphorylation of proteins 29a–c is consistent with the time course of platelet activation by thrombin and other agonists (2, 4, 10, 29), lending support to the potential relevance of these phosphorylation events in platelet signal transduction. The data presented identify proteins 29a–c as forms of HSP27, a low molecular weight member of the heat shock protein family.

The small heat shock proteins are ubiquitous and are more diverse than the larger heat shock proteins, differing in number between organisms and divergent in sequence from one organism to another (13). All share carboxyl-terminal homology with the lens structural protein α-crystallin and are able to form large intracellular polymeric aggregates, perhaps through interactions involving this region (13). Conversely, a recent report demonstrated that α B-crystallin is a member of the small heat shock protein family (35). The subcellular distribution of the small heat shock proteins varies, depending on cell type and perhaps as well on different degrees of phosphorylation (13, 36–38), though the functional significance of the different cellular dispositions of the proteins remains unclear.

HSP27 is known to be phosphorylated to several forms during normal cellular events, depending on stimulus and cell type (13, 36, 39–43). These studies, and the recognition of three distinct phosphorylated forms of HSP27 in human platelets, suggest that phosphorylation may regulate HSP27 function. In mammalian cells, varying degrees of HSP27 phosphorylation have been shown following stimulation by phorbol esters, calcium ionophore, or serum (36), as well as by the mitogens interleukin 1 and tumor necrosis factor (41, 42). However, despite previous pharmacologic attempts to explore HSP27 phosphorylation (12, 33, 36, 40, 42), and the recent identification of two phosphorylation sites in the murine small heat shock protein (44), the specific protein kinases involved in the phosphorylation of HSP27 remain unidentified.

The identity between HSP27 and the estrogen receptor-related 29-kDa protein P29 is also noteworthy. Coffer and colleagues (16, 30) first found P29 while preparing antibodies against the estrogen receptor. Although identification of P29

remained elusive (45), characteristics of the protein suggested to those investigators the application of P29 as a marker for estrogen responsiveness in tumor tissues (46). Working separately, McGuire and colleagues (47–49) had earlier identified a "24-kDa" protein as such a marker in breast cancer, then demonstrated the 24-kDa protein to be transcriptionally induced by estrogen, and more recently have shown this protein to be HSP27. Identification of the estrogen receptor-related protein as HSP27 strongly suggests that HSP27 is both transcriptionally induced by estrogen and interacts with the estrogen receptor, though the latter remains to be established. If so, this defines a potentially important regulatory loop in estrogen-responsive cells. The data also allow speculation regarding possible links between estrogen responsiveness and the cells that participate in hemostasis and thrombosis.

The specific role of HSP27 and its phosphorylation in platelet activation events is entirely unknown. Platelets do not have nuclei, display little protein synthetic function (50), and have yet to be demonstrated to be regulated by estrogen or other steroid hormones. Some possible roles for HSP27 in platelet activation include a chaperone function for an unidentified steroid receptor or other protein (14), interaction in a protooncogenic signal transduction pathway (11, 13, 51), participation in non-nuclear, steroid-mediated events (52), or direct participation of one or more of the HSP27 forms in the cytoskeletal, membrane, or secretory reorganizational events that occur with cell activation.

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