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Abnormal Cytoskeletal Assembly in Platelets from Uremic Patients

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The mechanisms involved in the hemostatic abnormality of uremic patients remain obscure. We have explored the response of normal and uremic platelets to surface activation at the ultrastructural level and analyzed changes in the composition of proteins associated with normal and uremic platelet cytoskeletons after stimulation with thrombin (0.01 and 0.1 U/ml). Cytoskeletons were obtained by extraction with Triton X-100, processed by sodium dodecylsulfate–polyacrylamide gel electrophoresis, and the presence of cytoskeletal proteins analyzed by densitometry. Under static conditions, uremic platelets spread with difficulty on formvar-coated grids. The percentage of platelets that spread fully on this polymer surface was statistically reduced compared with that of control platelets (11 ± 1.4 vs. 21 ± 1.6; $P < 0.05$). An impairment of cytoskeletal organization was observed in resting uremic platelets but abnormalities were more evident after thrombin activation. The incorporation of actin into the cytoskeletons of thrombin-stimulated uremic platelets was significantly reduced with respect to controls (6 ± 3% vs. 29 ± 5%; $P < 0.01$ after 0.01 U/ml and 28 ± 9% vs. 59 ± 10%; $P < 0.05$ after 0.1 U/ml). Decreased associations of actin-binding protein ($P < 0.01$), α-actinin ($P < 0.05$), and tropomyosin ($P < 0.05$) with the cytoskeletons of uremic platelets were also noted. No difference was observed for the incorporation of myosin into the cytoskeletons of activated uremic platelets. These results suggest functional and biochemical alterations of the platelet cytoskeleton in uremia, which may contribute to the impairment of platelet function observed in uremic patients. (Am J Pathol 1993, 143:823–831)

Activation of platelets depends on a series of receptor-mediated events that result in platelet secretion and aggregate formation. The actin content of resting platelets is predominantly in globular form (G-actin). Phosphorylation of proteins that follows platelet activation induces the polymerization of G-actin into filamentous actin (F-actin).[1] The molecular assembly of F-actin and organization of other structural proteins of the cytoskeleton are of critical importance for platelet shape change and internal contraction.[2–5]

Uremic patients have a bleeding tendency whose origins have not been well characterized.[6,7] Platelets from uremic patients show an impaired adhesion when exposed to glass beads[8] or to vascular surfaces when tested under flow conditions.[9–11] Despite evidence of the platelet adhesion defect, uremic patients are known to have normal or even increased levels of von Willebrand factor (vWF) in their plasma. Moreover, neither the presence of glycoprotein Ib or glycoprotein IIb-IIIa in the platelet membrane[12] nor the binding of vWF to these glycoproteins seems to be abnormal in platelets from uremic individuals.[11,13] Recent studies indicate that, despite the normal radioligand bindings to these GPs, spreading of uremic platelets on subendothelial surfaces is impaired in experiments performed with flowing blood.[14] The latter observation has suggested an abnormality of cytoskeletal function in platelets from uremic patients.

This study was designed in an attempt to compare the cytoskeletal assembly of normal and uremic platelets. For this purpose, the response of normal and

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uremic platelets to activation induced by an artificial surface was analyzed morphologically in the electron microscope and evaluated morphometrically. Changes induced in the association of cytoskeletal proteins of normal and uremic platelets subjected to thrombin stimulation were investigated by sodium dodecylsulfate–polyacrylamide gel electrophoresis and quantitated by densitometric scanning of the stained gels. Results suggest that defective assembly of the thrombin-activated platelet cytoskeleton may be related to abnormal spreading on surfaces and to prolonged bleeding in uremic patients.

Materials and Methods

Patients

The study, approved by the Human Experimental Committee of the Hospital Clínic, was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all the participants. Six uremic patients on maintenance hemodialysis (4 men and 2 women, age varying from 30 to 59 years) were included in this study. The six patients included in the study had previous history of bleeding. The bleeding symptoms registered in the clinical history of these patients included one or more of the following: epistaxis, easy bruising, gingival hemorrhages, menorrhagia, and hematuria. All the patients included in our study had difficulty in achieving correct hemostasis at the end of the dialysis procedure, requiring prolonged compression of the fistula access. Simplate bleeding times were prolonged in all the patients (range, 10 to >30 minutes; normal range, 2 to 8 minutes). All patients showed reduced aggregating response to 2 μM ADP and 1 mg/ml ristocetin (range of normality for maximal aggregation, 68 to 100% and 72 to 100%, respectively). Some patients also showed reduced response to 5 μg/ml collagen (range of normality, 75 to 100%). Hematocrit and hemoglobin levels in this group of uremic patients ranged from 23 to 29% and from 7.3 to 9.6 g/dl, respectively. Platelet counts and coagulation tests (prothrombin time, partial thromboplastin time, and fibrinogen levels) were within the normal range in each case. All patients received maintenance hemodialysis three times a week for more than 5 months. The patients were not hypertensive at the time that they were enrolled in the study. No patient had received blood-related products for 2 months, and none had taken aspirin or other drugs that affect platelet function for at least 2 weeks.

All six uremic patients included in this study were analyzed for both platelet spreading and alterations in the association of cytoskeletal proteins after thrombin activation. Results of these studies were compared with those obtained in six different healthy donors that were run in parallel.

Blood Sampling and Platelet Washing

Blood from uremic patients was always obtained just before a hemodialysis session. Platelet-rich plasma was separated by centrifugation of citrate-anticoagulated blood for 20 minutes at 100 × *g*. The concentration of citrate was adjusted (15 to 20 mM in plasma) so that free Ca^{2+} concentration was higher than 30 μM in both patients and controls. Platelets from platelet rich plasma were washed twice with equal volumes of citrate-citric acid-dextrose (93 mM sodium citrate, 7 mM citric acid, and 140 mM dextrose) pH 6.5 containing 5 mM adenosine and 3 mM theophylline.[15] The final pellet was resuspended in Hanks' balanced salt solution and incubated for 20 minutes at 37 C.

Aliquots of washed control and uremic platelet suspensions were used to analyze electrophoretically total platelet proteins. For this purpose, platelets were resuspended in 10 mM Tris-HCl, 0.15 M NaCl, and 5 mM *N*-ethylmaleimide (pH 7.0) and adjusted at 2 × 10^6/μL. A 1/5 volume of 12% (weight/volume) sodium dodecylsulfate was added and platelets were then solubilized by heating at 100 C for 5 minutes.

Thrombin-Induced Aggregation

Platelet aggregation studies were conducted in a Hitachi-Aggrecorder aggregometer. Samples of washed platelet suspensions from normal donors and uremic patients were adjusted at 150,000 platelets/μL and placed in 6-mm wide siliconized cuvettes. The minimum and maximum amplitudes of the recorder were respectively adjusted with platelet suspensions (0% transmission) and resuspension buffer (100% transmission). Fifty microliters of a 1-U/ml thrombin solution were added to 450 μL of platelet suspension, under stirring, to obtain a final concentration of 0.1 U/ml of thrombin. Results were expressed as percentages of maximal aggregation obtained after 5 minutes of stimulation.

Spreading Experiments

Fifty-microliter aliquots of washed platelet suspensions previously adjusted to 50,000 plts/μL were

layered on formvar-coated grids (mesh 200) and allowed to interact at 37 C for 20 minutes.[16] Grids were washed in Hanks' balanced salt solution. At the end of this time, any excess of platelets was removed by washing in Hanks' balanced salt solution. Platelets were then successively fixed for 30 minutes in 3% glutaraldehyde in 0.2 M cacodylate, pH 7.3, washed with distilled water, air dried, and examined in a Phillips 301 transmission electron microscope.

Morphometric Analysis of Surface-Activated Platelets

The surface equivalent to 10 randomly chosen grid squares was analyzed on two different grids prepared from the same patient or control donor. First, the total number of interacting platelets was determined on each grid square using low magnification. The state of activation of each platelet in the grid square was then morphologically classified according to previously established criteria.[17] In brief, platelets were classified as 1) early dendritic: platelets in an early state of spreading with several pseudopods extending radially from roughly spherical bodies (type I); 2) late dendritic: platelets in a more advanced state of activation with cytoplasm beginning to fill the spaces between pseudopods that are still evident (type II); and 3) fully spread: platelets in a final state of the process of activation whose cytoplasm has completely filled the spaces between pseudopods and whose central bodies have flattened into mounds or disappeared entirely (type III).

Obtention of Cytoskeletal Proteins

Platelet cytoskeletons were obtained according to the procedure described by Jennings et al[1] with minor modifications.[18] Samples of resuspended platelets were adjusted to 1.2×10^6 plts/μL and divided into three aliquots. One of them was left undisturbed at 37 C and the remaining two were subjected to stimulation with 0.01 and 0.1 U/ml thrombin, respectively. Samples were mixed by gentle inversion every 30 seconds. Ninety seconds after the activation was started, all the samples were treated with an equal volume of a lysis buffer containing 2% Triton X-100, 100 mM Tris-HCl, 10 mM ethylene glycol bis(β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid, and 4 mM ethylenediaminetetraacetic acid (pH 7.4). Cytoskeletal proteins in the Triton-insoluble residues were isolated immediately by sedimentation at 12,000 × *g* for 5 minutes at 4 C in a microfuge. The insoluble pellet was rinsed with lysis buffer diluted with an equal volume of washing buffer, without Triton X-100, and the suspension was centrifuged under the same conditions. The pellet was washed twice with washing buffer at 4 C. After removal of the supernatant, the pellet was solubilized with washing buffer containing 2% sodium dodecylsulfate and heated at 100 C for 5 minutes.

Desitometric Analysis of Total and Cytoskeletal Proteins

Samples of 50 μg of total platelet proteins or samples of the Triton-insoluble residues from equal number of thrombin nonactivated and activated platelets were applied to the gels. Total[19] or cytoskeletal proteins were separated by 7 to 12% sodium dodecylsulfate–polyacrylamide gel electrophoresis,[20] stained with Coomassie brilliant blue R250, and densitometrically quantified.[18,21] In our study, stained protein bands were densitometrically analyzed using digital-video technology provided by a computerized image analyzer running specific software (Elphor II; Biocom 200, Les Ulis Cedex, France).[22] Bands were manually selected on the monitor screen of the system. The software automatically scans the selected lane for protein bands, analyzes the color density of each protein band and integrates areas beneath densitometric peaks. To facilitate comparisons from gel to gel, the quantification of protein bands was performed under standardized conditions.

In the case of protein bands from whole platelet contents derived from control donors and uremic patients, the quantitation was performed on well characterized protein bands (actin-binding protein, myosin, GPIIb, and actin). These proteins were selected because they have characteristic molecular weights and separate into bands that are not masked by other proteins. Areas of these selected protein peaks in the lane containing platelet proteins from normal donors were calculated. Areas of the peaks corresponding to the same protein bands in lanes containing uremic platelet proteins were determined and referred to as percentages of the control.

In the case of cytoskeletal proteins, the area of each protein peak in the lane containing cytoskeletons extracted from nonstimulated platelets was calculated and considered one hundred per cent. Areas of the peak corresponding to the same protein band present in lanes containing cytoskeletons from

thrombin-activated platelets were determined. Association of certain protein with the thrombin-activated cytoskeleton was expressed as the percentage of increase over the amount of the same protein found in the respective lane containing non-activated platelets. Routine control experiments performed loading the lanes with decreasing amounts of cytoskeletal proteins extracted from control platelets have shown that the simple dilution of proteins samples does not affect the ratios calculated under the previously mentioned conditions.

A similar approach was used to compare amounts of proteins in cytoskeletons from resting control and uremic platelets. In this case, areas of the peaks corresponding to the same protein bands present in lanes from uremic platelets were determined and referred as percentages of the respective control.

Statistics

Means and SE were determined from the previous results. Student's *t*-test was used for statistical comparisons. A $P < 0.05$ was considered statistically significant.

Results

Response of Washed Platelets to Thrombin

Maximum aggregation induced observed on washed platelets from the control group exposed for 5 minutes to 0.1 U/ml thrombin was 78.5 ± 3.7% (mean ± SE; range, 65 to 91%). Under the same experimental conditions the aggregating response in the group of uremic patients was slightly decreased (69 ± 3.5%; range, 61 to 85%). However, differences between patients and controls never reached the levels of statistical significance.

Experiments on Surface-Activated Platelets

After 20 minutes of exposure to the formvar surface, the average number of platelets per grid square in the control experiments was 40.8 ± 3.3 (mean ± SE). When interacting platelets were classified according to their stage of activation, 34 ± 4% of these platelets were found as early dendritic, 43 ± 3.5% consisted of late dendritic forms, and the remaining 21 ± 1.6% were constituted by fully spread platelets.

The total number of platelets attached per grid square was slightly lower in experiments performed with uremic platelets (38.6 ± 3.3), but differences never reached the levels of statistical significance. As is shown in Figure 1, uremic platelets spread with difficulty on the formvar surface. The morphometric quantification of the interacting platelets showed 40 ± 4.1% of platelets in early dendritic form; 46 ± 3.4% had reached late dendritic stages and only 11 ± 1.4% had achieved full spreading (type III) on the formvar surface ($P < 0.01$ vs. control platelets). Figure 2 illustrates the morphological differences between control and uremic platelets.

Analysis of Total Platelet Proteins

Electrophoretic studies in one-dimensional sodium dodecylsulfate–polyacrylamide slab gels stained with Coomassie blue did not show any differences between profiles corresponding to control and uremic whole platelet proteins (Figure 3). Quantitation of densitometric peaks corresponding to a group of major protein bands demonstrated that these proteins were present in uremic platelets at similar levels than those observed in the corresponding controls. Densities for actin-binding protein in lanes from uremic platelets ranged from 85.2 to 110.1% of the values observed in controls. Values calculated for the remaining analyzed bands in uremic platelets ranged from 62.6 to 138.6% for myosin, from 79.8 to 125.9% for GPIIb, and from 71.9 to 118.6% in the case of actin. The range of densitometric values calculated for these selected bands in uremic platelets was indistinguishable from that calculated for the same bands in control platelets.

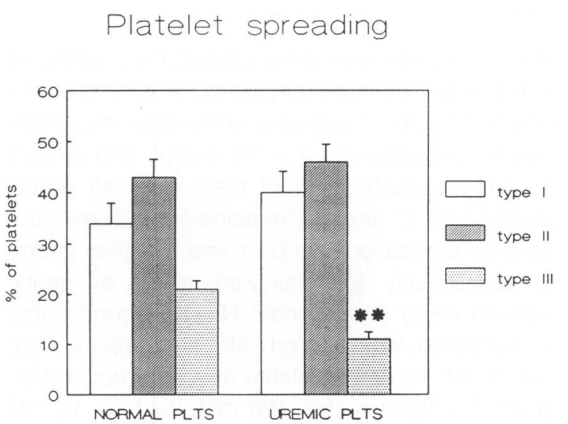

Figure 1. *Bars showing different stages of platelet activation observed in normal individuals ($n = 6$) or uremic patients ($n = 6$). Values represent percentages (mean ± SE) of platelets in each stage of activation. Asterisks denote statistical significance with respect to controls (** $P < 0.01$).*

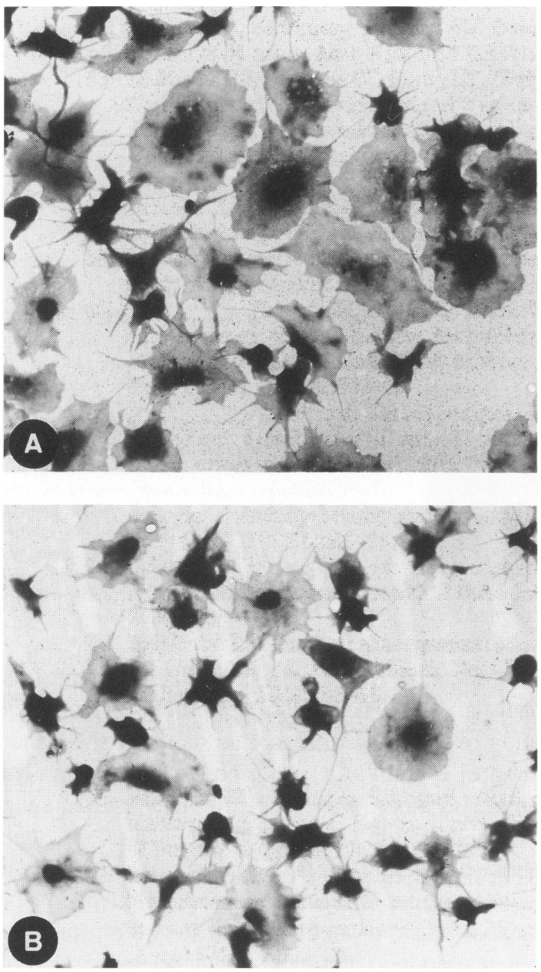

Figure 2. *Morphological aspect of platelets interacting with formvar-coated grids. Control platelets obtained from a normal donor* **(A)** *are observed in advanced stages of spreading, whereas most of the platelets from a uremic patient* **(B)** *remain in dendritic forms.*

Analysis of Cytoskeletal Proteins

The presence of cytoskeletal proteins retained in the Triton-insoluble pellets from uremic platelets was usually decreased with respect to values observed in controls (Figure 4, see lane 5 vs. 1). Amounts of proteins found in cytoskeletons from resting uremic platelets accounted for 69 to 90% of those recovered from the identical number of control platelets. Despite the differences, reductions were only statistically significant for actin-binding protein (83 ± 3.12, mean ± SE, $P < 0.05$) and actin (81 ± 3.78, $P < 0.05$).

Thrombin stimulation resulted in an increased incorporation of proteins with the Triton X-100–insoluble fractions collected from either control or uremic platelets (Figure 4). The densitometric evaluation of the stained gels obtained from control platelets showed an increase in the association of actin-binding protein, myosin, α-actinin, actin, and tropomyosin to the Triton X-100–insoluble cytoskeletons after thrombin stimulation. The association of cytoskeletal proteins was more evident in platelets stimulated with a higher concentration of thrombin (Figure 5).

Association of cytoskeletal proteins with the Triton-extracted residues in uremic platelets was markedly altered after stimulation with both concentrations of thrombin (0.01 and 0.1 U/ml). The densitometric evaluation performed after stimulation with 0.01 U/ml thrombin showed a statistically significant decrease in the incorporation of actin-binding protein ($P < 0.01$ vs. normal platelets), actin ($P < 0.01$ vs. normal platelets), and tropomyosin ($P < 0.05$ vs. normal platelets). Alterations of the cytoskeletal assembly were also evident in uremic platelets activated with 0.1 U/ml thrombin. With this higher concentration, marked reductions in the incorporation of actin-binding protein ($P < 0.01$), α-actinin ($P < 0.05$), actin ($P < 0.05$), and tropomyosin ($P < 0.01$) were noted when densitometric results obtained in uremic platelets were compared with those of normal platelets (Figure 5). The association of myosin with cytoskeletons of uremic platelets activated with both concentrations of thrombin was similar to that found in normal platelets.

Discussion

The existence of a hemostatic defect in patients with uremia is well known, but the precise cause of bleeding symptoms has remained obscure. Morphological studies in this investigation have shown a platelet-spreading defect in six uremic patients with a well-documented previous history of excessive bleeding. After exposure to formvar grids under conditions that cause most normal cells to change into late dendritic or spread forms, uremic platelets remained dendritic. Biochemical evaluation of cytoskeletal organization in resting uremic platelets revealed decreased proportions of actin-binding protein and actin, which might relate with failure to spread. Cytoskeletons of thrombin-activated uremic platelets contained significantly less actin-binding protein, α-actinin, actin, and tropomyosin than normal cytoskeletons, although the amount of myosin incorporated was the same. These results are consistent with functional and biochemical alterations of the platelet cytoskeleton in uremia, which may be related to defective hemostasis.

Mechanisms involved in the spreading of washed platelets on artificial surfaces are not precisely un-

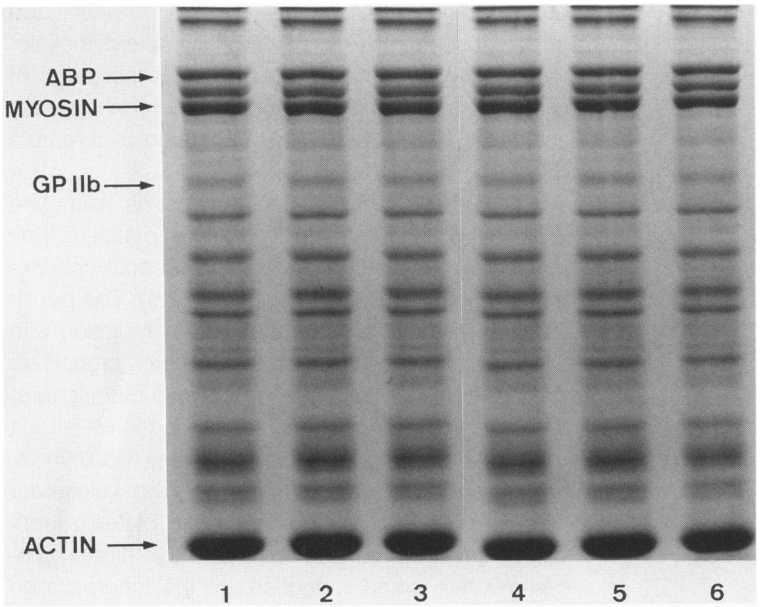

Figure 3. *Sodium dodecylsulfate–polyacrylamide gel electrophoresis of polypeptides present in control and uremic platelets after staining with Coomassie blue. Sodium dodecylsulfate-soluble platelet extracts (50 μg of protein) were separated on single dimension 7- to 12-% acrylamide gradient slab gels in the absence of disulphide reduction. Profiles of three uremic patients (lanes 2, 4, and 6) are illustrated together with those of the control donors (lanes 1, 3, and 5) investigated in parallel. Electrophoretic patterns of platelet polypeptides from control donors or uremic patients are practically indistinguishable. ABP, actin-binding protein; GPIIb, glycoprotein IIb.*

derstood. Spreading of platelets on formvar-coated surfaces is not dependent on GPIb or GPIIb-IIIa.[23] In contrast, exposure of platelets to cytochalasins effectively inhibits the active spreading of platelets on this artificial surface.[24–26] The latter data indicate that spreading of platelets under such conditions is mainly dependent on platelet cytoskeletal assembly. Results of this study demonstrate that the progression of spreading by uremic platelets on a foreign surface is defective, thus suggesting an altered cytoskeletal assembly.

Platelets possess two well differentiated cytoskeletons, the membrane and the cytoplasmic skeletons.[3,5,27] Actin is a constituent of both of them. Assembly of F-actin and association of these filaments with cytoplasmic myosin during platelet activation is known to play a critical role in platelet shape change and internal contraction.[2,28,29] The densitometric studies performed on regular sodium dodecylsulfate–polyacrylamide gel electrophoresis of whole platelets (not detergent extracted) from the uremic patients enrolled in our study indicated that total polypeptide contents were similar to those observed in normal donors. Contrasting with the latter results, we observed decreased contents of actin-binding protein and actin in detergent-resistant cytoplasmic cytoskeletons obtained from equal number of resting uremic platelets. The latter results suggest that the basic organization of the cytoskeleton is already deficient in resting uremic platelets.

The conversion of G-actin into F-actin was clearly impaired in uremic platelets that were subjected to thrombin activation. Although association of myosin with the uremic cytoskeletons was normal after activation, abnormalities in the generation of contractile forces might be expected due to decreased interaction of F-actin with phosphorylated myosin. The decreased incorporation of contractile proteins into the cytoskeleton of thrombin-activated platelets cannot be merely explained by slight reductions in the amounts of proteins in cytoskeleton of resting platelets. Control experiments in which proteins from resting and thrombin-activated cytoskeletons from normal platelets were diluted to the same extent showed no relevant changes in the percentages of association of the proteins analyzed in our studies.

Actin-binding protein has been identified as the protein linking the membrane skeleton to GPIb-IX, GPIa, GPIIa, and possibly to other glycoproteins.[30,31] It has been suggested that this association regulates the ability of GPIb to bind vWF.[27] Establishment of transmembrane linkage between surface-ligand complexes and the force-generating contractile elements of the cytoplasmic cytoskeletons is of crucial importance for effective platelet function.[30–33] The finding of defective association of actin-binding protein and actin in cytoskeletons from resting or activated uremic platelets observed in our studies would be compatible with a defective connection between cytoplasmic and membrane cytoskeletons. These results may explain the different morphologies of uremic platelets during spreading on artificial surfaces found in our studies. Moreover, other cytoskeletal proteins of the platelet cytoplasm, such as α-actinin and tropomyosin,

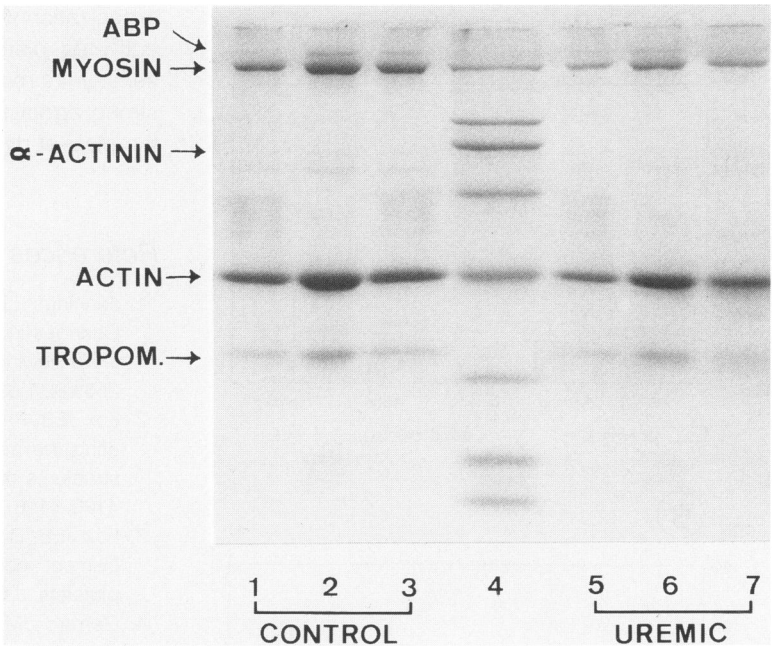

Figure 4. *Sodium dodecylsulfate–polyacrylamide gel electrophoresis of Triton-insoluble platelet cytoskeletons from a normal donor and a uremic patient before and 90 seconds after thrombin stimulation. Lanes: 1) unstimulated platelets from a control, 2) control platelets after 0.1 U thrombin, 3) control platelets after 0.01 U thrombin, 4) molecular weight standards,* 5) unstimulated uremic platelets, 6) *uremic platelets after 0.1 U thrombin, and 7) uremic platelets after 0.01 U thrombin. Abnormalities in actin-binding protein (ABP), myosin, actin, and tropomyosin (tropom) bands of this patient are apparent when lanes containing platelets activated with low concentrations of thrombin (lane 7) are compared with similarly activated control platelets (lane 3).*

known to play a role in the linkage of actin filaments to membrane[4,30,31] and in the regulation of interactions of these filaments with other proteins,[2] were found to be decreased in thrombin-activated uremic platelets.

Previous studies from our group have shown the existence of a defective interaction of uremic platelets with subendothelial surfaces.[9,10,12] Further evidence in the current literature has also confirmed our previous findings.[11,34] A recent study has suggested that the primary attachment of uremic platelets to the subendothelial surface is well preserved, but the spreading capabilities of affected platelets is impaired.[14] The spreading defect of uremic platelets would be functionally similar to that observed in severe Glanzmann's thrombasthenia.[35,36] Fujimura et al[37] who recently analyzed the cytoskeletal assembly in activated thrombasthenic platelets reported normal myosin assembly, decreased incorporation of actin-binding protein and tropomyosin, and actin and α-actinin incorporations up to the lower limits of the normal range. The defect we have noticed in the cytoskeletal organization of thrombin-activated uremic platelets resembles that observed in thrombasthenic platelets. Despite similarities, a word of caution is required because severe Glanzmann's patients congenitally lack GPIIb-IIIa, whereas uremic patients possess quantitatively normal GPIIb-IIIa in their platelets. In any case, the abnormal cytoskeletal assembly found in our studies may help to explain the persistence of an adhesion defect in uremic platelets noted in several studies,[8–11] despite normal presence[12] and normal binding functions of membrane receptors found in the recent literature.[11,13]

The impairment in the cytoskeletal assembly found in activated uremic platelets may reflect abnormalities of the thrombin receptor. However, the response of uremic platelets to thrombin in our aggregation tests did not differ from that of controls used in our studies. The latter results would be in agreement with those reported by Zwaginga et al.[34] Unfortunately, aggregometric techniques have inherent limitations to detect mild defects of platelet function, especially when strong agonists are used. Thus, we believe that an abnormality of the thrombin receptor or in the stimulus-response coupling in uremic platelets cannot be ruled out on the basis of the aggregation data.

Toxins present in uremic plasma thought to impair platelet adhesive functions[9,34] could interfere with the biochemical events taking place during cytoskeletal assembly that follows platelet activation. We have made several unsuccessful attempts to reproduce the cytoskeletal abnormalities observed in uremic platelets by incubating normal platelets in uremic plasma for up to 6 hours. More extended periods might be required, but under such conditions it has been extremely difficult to determine whether alterations observed were related to the toxics added or to the development of platelet storage lesions.

Repeated activation of platelet by dyalisis procedures[38] may result in alternate cycles of poly/

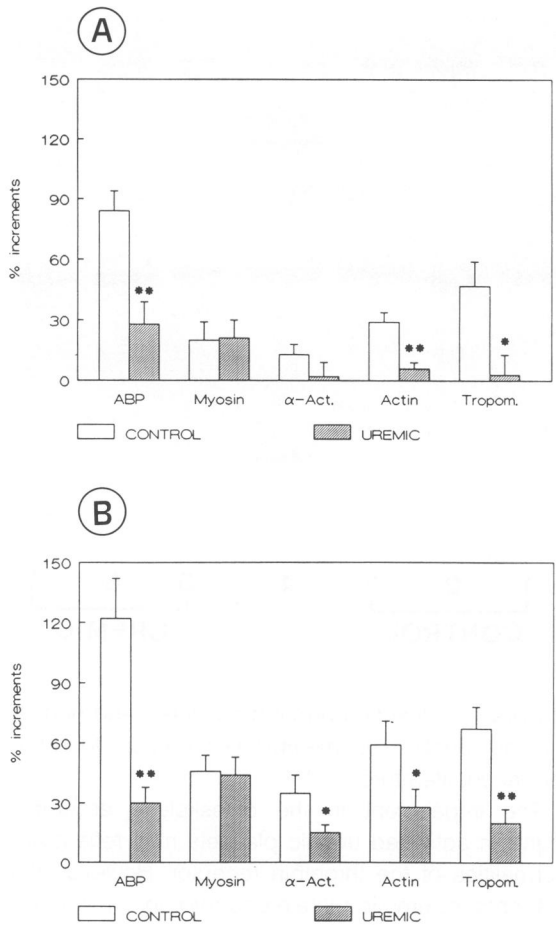

Figure 5. *Bars showing percentages of increase in the association of cytoskeletal proteins as calculated by scan densitometry of the electrophoretic gels. Bars display results (mean ± SE) obtained in control (empty bars) and uremic (dashed bars) cytoskeletons after 0.01 U/ml (A) or 0.1 U/ml of thrombin (B). Values are expressed as percentages of increase over the amount of the same protein found in the cytoskeletons of nonactivated platelets. Triton-insoluble cytoskeletons analyzed were derived from the same individuals involved in spreading experiments. Amounts of actin-binding protein (ABP), α-actinin (α-Act), actin, and tropomyosin (tropom) bound into uremic platelet cytoskeleton are significantly less than those incorporated by control cells, except for myosin. Asterisks denote statistical significance with respect to controls (* $P < 0.05$; ** $P < 0.01$).*

depolymerization of cytoskeletal proteins. It remains to be established how many cycles of activation-deactivation can a platelet undergo before developing refractoriness.[39] Studies are in progress to investigate the cytoskeletal assembly in platelets from patients subjected to peritoneal dyalisis or in patients at end-stage chronic renal failure before and after starting hemodialysis. Results of these studies will help to resolve the contribution of dialysis procedures to the cytoskeletal alterations we have referred.

In summary, our results support the concept that platelets from uremic patients fail to spread fully after surface activation. An impairment of cytoskeletal organization was observed in resting and thrombin-activated platelets. The abnormal cytoskeletal assembly in response to activation by surfaces or strong agonists in suspension may help to explain the platelet dysfunction observed in uremia.

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