Movement of fibrinogen receptors on the ventral membrane of spreading platelets

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Abstract

We have used fibrinogen–gold conjugates and fluorescein-labelled fibrinogen to examine the presence of fibrinogen receptors on the ventral membrane of spreading platelets and to visualize the redistribution of the receptors during platelet spreading. The movement of fibrinogen–gold conjugates was observed in real time using video-enhanced interference reflection microscopy (VEIRM) and the redistribution of fluorescein-labelled fibrinogen was examined using video-intensified epifluorescence microscopy (VIFM). Fibrinogen was picked up by spreading platelets and moved towards the centre of the platelets. The velocity of the movement of fibrinogen–gold conjugates under the ventral membrane ranged from less than $0.1 \, \mu m \, min^{-1}$ to more than $10 \, \mu m \, min^{-1}$. The average velocity in the peripheral web region was more than $6 \, \mu m \, min^{-1}$, and was reduced to around $1 \, \mu m \, min^{-1}$ in the outer and inner filamentous zones. The centralized fibrinogen molecules were internalized and eventually removed from the platelets. Video microscopy in conjunction with colloidal gold particles provides a simple method for real-time observation of the movement of fibrinogen receptors on the ventral membrane of spreading platelets. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Fibrinogen–gold conjugates; Colloidal gold; Fibrinogen receptors; Platelets; Video microscopy; Interference reflection microscopy

1. Introduction

Platelet activation on biomaterials presents one of the major problems in the development of blood-compatible prosthetic devices. Platelet adhesion and activation on biomaterials depends largely on the presence of fibrinogen on the surface [1, 2]. When platelets contact the fibrinogen-coated surface, the round shape undergoes a remarkable transformation to a fully spread form which is flattened against the surface. Platelet spreading is accompanied by intracellular reorganization of a three-dimensional cytoskeletal network and associated proteins [3–6]. The mechanisms by which external contact with surface-adsorbed fibrinogen molecules can trigger platelet spreading and cytoskeletal reorganization is poorly understood. One approach would be to examine the mode of fibrinogen interaction with their receptors on the platelet membrane.

The interaction of fibrinogen in solution with receptors on the dorsal membrane of spreading platelets has been investigated by many investigators [7, 8]. It has been shown that the binding of fibrinogen to receptors on the dorsal membrane of spreading platelets causes redistribution of fibrinogen-receptor complexes to the central granulomere region [9]. Compared to the extensive research on the movement of fibrinogen receptors on the platelet dorsal membrane, the study on the interaction of surface-adsorbed fibrinogen with their receptors on the ventral membrane of spreading platelets has been rare.

Up until a decade ago, the presence of fibrinogen receptors on the ventral membrane of spreading platelets was not clearly shown. Feuerstein and Kush [10] observed for the first time that the spreading platelets modified the surface-bound fibrinogen. Using epifluorescence video microscopy, Feuerstein and Kush [10] clearly showed that the surface-adsorbed fibrinogen was removed from the surface by the spreading platelets. It was not clear, however, how the preadsorbed fibrinogen was redistributed by platelets. Several years ago the movement of surface-adsorbed fibrinogen on the platelet

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ventral membrane was confirmed for the first time using fibrinogen–gold conjugates [11, 12].

In this study, we used fibrinogen–gold conjugates and fluorescein-labelled fibrinogen to examine the presence of fibrinogen receptors on the platelet ventral membrane and to visualize the dynamic redistribution of the receptors during platelet spreading. The movement of fibrinogen–gold conjugates was observed in real time using video-enhanced interference reflection microscopy and the redistribution of fluorescein-labelled fibrinogen was examined using video-intensified epifluoresence microscopy.

2. Observation of fibrinogen–gold conjugates using video-enhanced interference reflection microscopy

Recently, video microscopy coupled with digital image processing has been used to visualize objects many times smaller than the Raleigh resolution limit of 0.2 μm [13–16]. Any particle, regardless of its absolute size, is theoretically detectable in a light microscope as long as it produces sufficient contrast at the image level [15, 17]. Individual colloidal gold particles as small as 5 nm were visualized using video microscopy [17–19]. While the size of the gold particles is well below the resolution of the light optics, individual particles or small clusters can be observed in a video microscope via their inflated diffraction image [20]. Colloidal gold particles in different sizes appear on the screen with the same apparent size of about 260 nm [19]. Since the limit of resolution of a light microscope is 200 nm, particles which are separated by less than 200 nm appear as a single dot. The movement of individual particles, however, can be accurately determined by the frame-by-frame difference between the positions of a moving particle and a stationary reference particle. This differential measurement of particle position eliminates the effect of various factors that affect the field as a whole [16]. Thus, the precision of position measurements by a light microscope is not limited by the resolution of the light microscope [16].

We have used video-enhanced interference reflection microscopy (VEIRM) to visualize protein–gold conjugates. The most significant advantage of using VEIRM is that protein–gold conjugates located under the ventral membrane can be visualized and easily distinguished from those on the dorsal membrane. In VEIRM, protein–gold conjugates at the top membrane of spread platelets appear white (black arrow in Fig. 1A), while those under the ventral membrane appear black (white arrow in Fig. 1A). Protein–gold conjugates on the upper platelet membrane appear as black dots in video-enhanced brightfield microscopy (VEBM) (Fig. 1B). The VEBM, however, is not easy to visualize gold labels located under the ventral membrane of platelets.
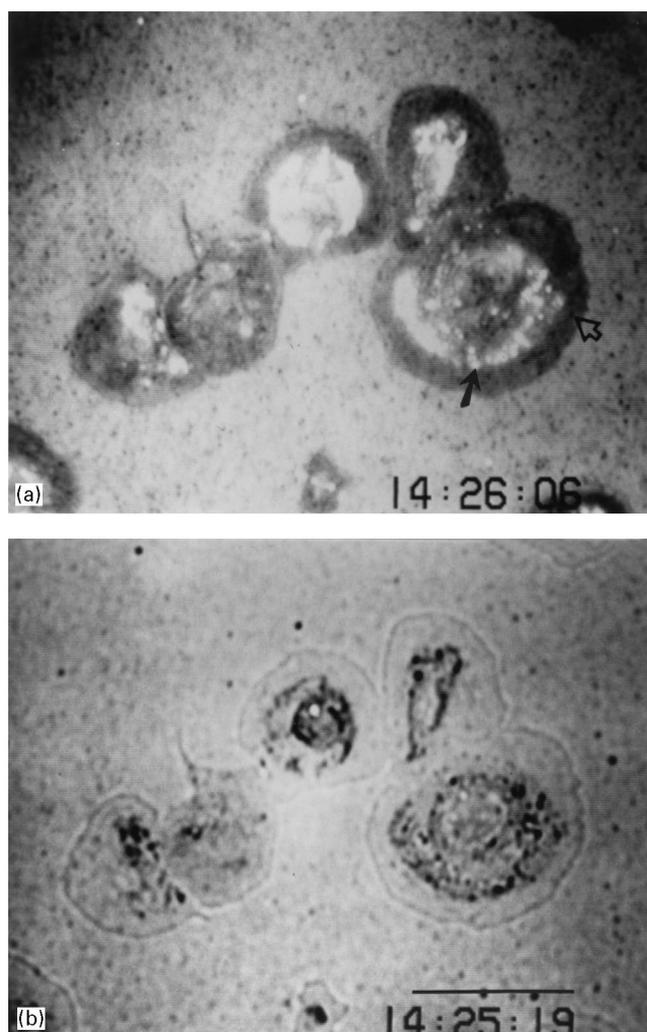

Fig. 1. (A) Video-enhanced IRM picture of fibrinogen–gold conjugates on the dorsal and ventral membranes of spread platelets. The white areas represent clusters of fibrinogen–gold conjugates on the dorsal membrane. Fibrinogen–gold conjugates on DDS–glass surface and on the platelet ventral membrane appear as black dots. (B) Video-enhanced brightfield microscopic picture of fibrinogen–gold conjugates on the dorsal membrane of spread platelets. The conjugates appear black. Platelets were allowed to spread on DDS–glass for 45 min at room temperature.

The VEIRM of fibrinogen–gold allowed continuous observation of the movement of fibrinogen molecules throughout various stages of platelet spreading. This was an advantage over video-intensified fluorescence microscopy (VIFM) which required fluorescence probe-labelled fibrinogen. VIFM could not allow continuous observation of fibrinogen due to photobleaching of the fluorescence probe. VIFM, however, was useful in monitoring the localization of fibrinogen molecules which were taken up by platelets (see Results). The combination of VEIRM and VIFM made it possible to track the pathways of fibrinogen molecules after their interaction with receptors on the platelet membrane.

3. Materials and methods

3.1. Protein preparation

Commercially obtained human fibrinogen (Sigma, Type I, St Louis, MO, USA) was purified further by the Laki method [21] as described previously [22]. The purified fibrinogen showed clottability of at least 97%. The fibrinogen in phosphate-buffered saline without divalent cations (PBS, pH 7.4) was frozen in aliquots at $-70°C$ at a concentration of about 2.0 mg ml^{-1}. Human albumin (Sigma, 1X crystallized) was used as received after dissolving in PBS. The concentrations of both proteins were monitored by absorbance at 280 nm using absorptivities of 1.506 and 0.58 for 0.1% solutions of fibrinogen and albumin, respectively.

3.2. Preparation of protein–gold conjugates

Colloidal gold particles with an average diameter of 18 nm were prepared following the procedure described previously [23–25]. The pH of the colloidal gold solution at room temperature was 5.7 as measured using a gel-filled combination electrode (Orion, Cambridge, MA, USA). The absorbance of the prepared colloidal gold solution at 525 nm was 0.93 and this corresponded to 8.30×10^{11} particles ml^{-1} [24]. The colloidal solution was cooled and stored at 4°C until use.

Fibrinogen–gold and albumin–gold conjugates were prepared by adding 0.2 ml of protein solution (0.1 mg ml^{-1} for fibrinogen and 0.3 mg ml^{-1} for albumin) to 2 ml of colloidal gold solution. The pH of the solution was maintained at 5.7 for albumin and 6.7 for fibrinogen to obtain the most stable protein–gold conjugates [25]. After 30 min at room temperature, 0.1 ml of 1% poly (ethylene glycol) (M_w 20 000) solution was added for further stabilization. The protein–gold conjugates were separated from the bulk solution by centrifuging (maximum centrifugal force of 12 100 g) for 30 min in an angle head rotor (Beckman, Model J2-21 M/E, Palo Alto, CA, USA). The sedimented particles were collected and resuspended in desired solutions.

3.3. Preparation of fluorescein-labelled proteins

Fluorescein isothiocyanate (FITC, Sigma) was dissolved in PBS and the solution was filtered through a 0.2 μm filter (Millipore). The concentration of FITC was determined by measuring the absorbance at 495 nm. The absorptivity used was 206 for 0.1% (or 1 mg ml^{-1}) solution. To 1 ml of protein solution (1.2 mg ml^{-1}) was added 1 ml of FITC solution (0.16 mg ml^{-1}). The solution was gently mixed and left at room temperature in the dark for up to 24 h. The fluorescein-labelled protein was separated from free FITC using a desalting column (Bio-Gel P-6DG, Bio-Rad).

3.4. Surfaces

Glass slides (Fisher) and glass coverslips ($1'' \times 3''$, #1–1/2, Bellco) were cleaned using IsoClean (IsoLab, Akron, OH, USA). They were soaked in 2% IsoClean solution at 60°C for 3 h and washed extensively with distilled water. They were then soaked again in deionized distilled water at 60°C for 3 h. Finally, they were washed with plenty of deionized distilled water and dried at 80°C. For the coating of glass coverslips with dimethyldichlorosilane (DDS, Aldrich), clean glass coverslips were immersed in a 5% solution of DDS in chloroform for 30 min. The treated coverslips were rinsed with chloroform and ethanol in sequence twice. Finally, the DDS-coated coverslips were washed in deionized distilled water and dried at 80°C.

3.5. Perfusion chamber

A simple perfusion chamber was devised to coat the glass coverslips with proteins and to introduce platelets. The chamber was assembled with a glass slide ($1'' \times 3''$), a glass coverslip, and a silicone rubber sheet (0.005'' thickness, Dow Corning, Midland, MI, USA) as a spacer [5]. The length of a glass slide was reduced to 2.8'' by cutting off one end of the slide using a diamond knife. The silicone rubber sheet was treated lightly with silicone grease and placed between glass slide and coverslip. The three pieces were held together by pressing for several seconds. The perfusion chamber was made immediately before experiment. The PBS was added into the perfusion chamber by placing the end of a pipette tip on to one end of the glass slide. The other end of the glass slide received a filter paper wick or a folded Kimwipe that drew the solution out from the perfusion chamber. All other solutions were added in this way.

3.6. Adsorption of protein–gold conjugates

The prepared protein–gold conjugates were introduced to the surface using a perfusion chamber. The buffer solution of the perfusion chamber was replaced with the protein–gold conjugates. Protein–gold conjugates at the bulk concentration of 8.51×10^{11} ml^{-1} (Abs = 1.0 at 525 nm) [24, 25] were allowed to adsorb onto the surface for 2 min. After this time, the number of conjugates on the surface was high enough to see the redistribution of the conjugates during platelet spreading. The adsorption of colloidal–gold conjugates onto the surface was visualized by VEIRM. After the adsorption was complete, the unadsorbed colloidal–gold conjugates were washed away using buffer.

3.7. Protein adsorption

Protein solution was added to the chamber by replacing PBS. The bulk protein concentration was varied

from 0.1 to 50 mg ml^{-1} for albumin, and from 0.001 to 1 mg ml^{-1} for fibrinogen. After a predetermined time, the chamber was washed with PBS to remove unadsorbed protein.

3.8. Platelet preparation

Blood was withdrawn from healthy adult volunteers by venous puncture after informed consent. Blood (20 ml) was collected into Vacutainers (Becton Dickinson, Rutherford, NJ, USA) containing buffered sodium citrate and mixed by gentle inversion. Platelet-rich plasma (PRP) was separated from whole blood by centrifugation at 100 g for 20 min at room temperature. Citrated PRP was then incubated in a 37°C water bath for 30 min. Platelets were isolated from plasma proteins by passage through a Sepharose CL-2B column (2.5 × 10 cm) which was pre-equilibrated with PBS. The appearance of platelets was monitored by a UV-detector (Gilson, Middleton, WI, USA). The platelet peak was separated from the protein peak. The platelet suspension obtained was kept at room temperature and used in less than 30 min after separation.

3.9. Video microscopy

Platelets in PBS were introduced into the perfusion chamber and allowed to adhere to the surface at room temperature. The video microscopy used in this study was described in detail in our previous publications [5]. The video cameras used were an instrumentation grade Newvicon camera (Model 65, Dage-MTI, Michigan City, IN, USA) for brightfield and interference reflection microscopic images, and a low-light-level camera with a silicone-intensifier target tube (Model 66, Dage-MTI) for fluorescence images. The level of photon noise with the low-light-level camera was so high that at least 10 VIFM images were averaged using a digital image processor (PC Consulting). Image averaging removed most of the noise which results from using a low-light-level camera. All real-time images with information on time were recorded on a video tape. Those images were used to measure the velocities of the movement of fibrinogen–gold conjugates under the ventral membrane of spreading platelets.

4. Results

4.1. The movement of fibrinogen–gold conjugates

As shown in Fig. 1, the colloidal gold particles which are present on the dorsal membrane of spreading platelets appear white while those on the ventral membrane appear black by VEIRM. This makes it possible to know whether the colloidal gold particles are moving on the
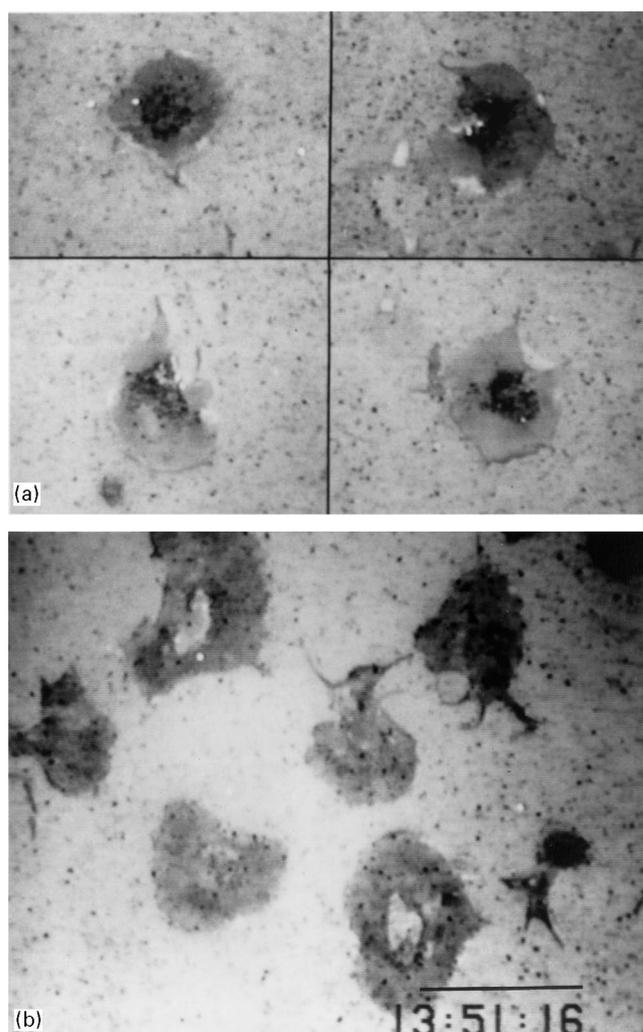

Fig. 2. Video-enhanced IRM pictures of platelets spread on glass coated with fibrinogen–gold (A) and albumin–gold conjugates. The fibrinogen–gold conjugates were clustered at the centre of spread platelets (A), while albumin–gold conjugates remained in their initial position during platelet spreading (B). Platelets were allowed to spread on glass for 30 min at room temperature.

dorsal or the ventral membrane of spreading platelets. When the protein–gold conjugates (both fibrinogen–gold and albumin–gold conjugates) were adsorbed onto the glass surface, they were distributed homogeneously on the surface. Platelets were allowed to settle on the surface precoated with protein–gold conjugates. Fig. 2 shows platelets which were adhered and spread on the surface coated with fibrinogen–gold conjugates (Fig. 2A) or with albumin–gold conjugates (Fig. 2B). As shown in Fig. 2A, fibrinogen–gold conjugates were clustered to the centre of the spread platelets. On the other hand, albumin–gold conjugates did not move from their initial positions on the surface. Fig. 2B shows albumin–gold conjugates which are still remaining in the peripheral web region of the spread platelets. Albumin–gold conjugates were not clustered or transferred towards the centre of spreading
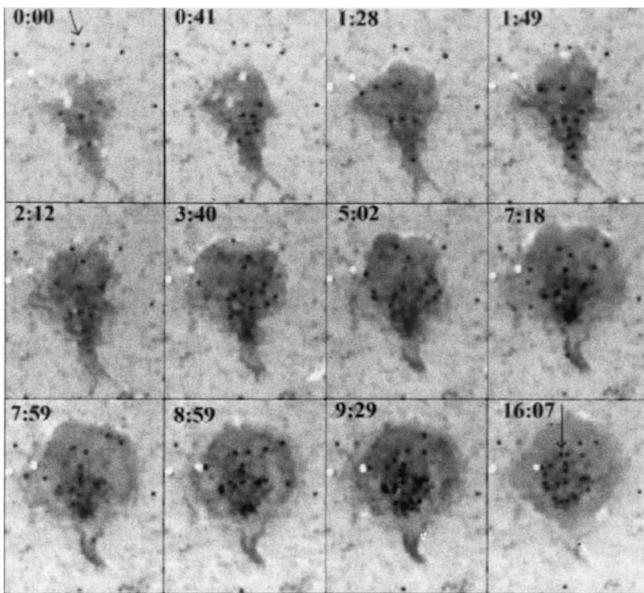
Fig. 3. Video-enhanced IRM images showing a sequence of platelet spreading and the resultant centripetal redistribution of fibrinogen–gold conjugates on the ventral membrane. The centripetal movement of the two selected fibrinogen–gold conjugates (indicated by arrows at times 0:00 and 16:07 min) can be traced. Some fibrinogen–gold conjugates shown here are highlighted to make it easier to show the centripetal movement. Each panel of the sequence is 10 μm wide.
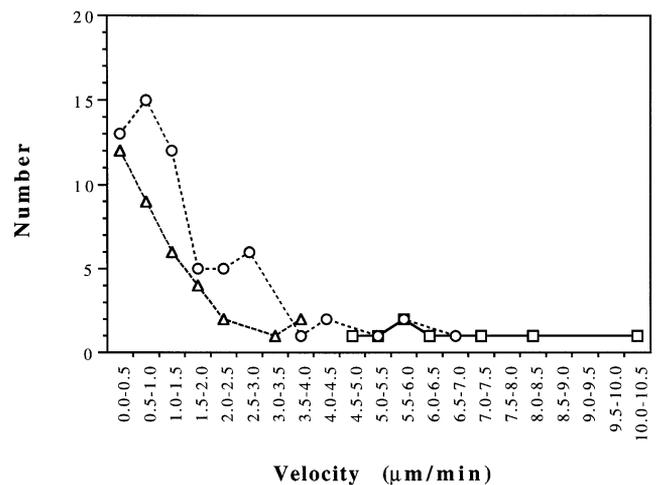

Fig. 4. The distribution of velocities of fibrinogen–gold conjugates on the ventral membranes of spreading platelets. The movement of fibrinogen–gold conjugates were measured in the peripheral web (□), outer filamentous zone (○) and inner filamentous zone (△). The average velocities of fibrinogen–gold conjugates in the peripheral web, outer filamentous zone and inner filamentous zone were 6.51 ± 1.63, 1.51 ± 1.32 and 1.04 ± 0.96 $\mu m \, min^{-1}$, respectively.

platelets. They remained on the same spots during platelet spreading. Thus, the movement is a salient feature of fibrinogen–gold conjugates only, and this implies the movement of the fibrinogen receptors on the ventral membrane of spreading platelets.

To appreciate the movement of the fibrinogen–gold conjugates under the ventral membrane of spreading platelets, the redistribution of pre-adsorbed fibrinogen–gold conjugates during spreading of a selected platelet was examined at timed intervals. Fig. 3 shows an example of the sequence of the redistribution of fibrinogen–gold conjugates during platelet spreading. The movement of two selected fibrinogen–gold conjugates (indicated by the arrows at times 0:00 and 16:07 min in Fig. 3) towards the centre of the platelet is clearly seen. Once the fibrinogen–gold conjugates interacted with the fibrinogen receptors, they were transferred towards the centre of a spreading platelet. The movement of the conjugates suggests that they are detached from the surface and move in conjunction with the movement of fibrinogen receptors on the platelet membrane.

Velocities of the centripetal movement of the fibrinogen–gold conjugates taken up by the platelet were measured using recorded video images. Fig. 4 shows the distribution of velocities of fibrinogen–gold conjugates moving on the ventral membrane of spreading platelets. The movement of more than 100 fibrinogen–gold conjugates was measured on 20 different samples. As shown in

Fig. 4, most of the conjugates were observed in the inner and outer filamentous regions. The velocity of the centripetal movement of the fibrinogen–gold conjugates ranged from less than $0.1 \, \mu m \, min^{-1}$ to more than $10 \, \mu m \, min^{-1}$. Fig. 4 clearly shows that the velocity varied depending on the location of conjugates on the platelet. The velocity was highest in the peripheral web region with the average velocity of $6.51 \pm 1.63 \, \mu m \, min^{-1}$, and sharply decreased in the filamentous zones. The movement of the conjugates in the outer filamentous zone was faster than that in the inner filamentous zone. The average velocities in the outer and inner filamentous zones were 1.51 ± 1.32 and $1.04 \pm 0.96 \, \mu m \, min^{-1}$, respectively. At these speeds it takes about 10 min for fibrinogen–gold conjugates at the peripheral web region to migrate to the centre of the spread platelet. In our experiments, the most noticeable clustering of fibrinogen–gold conjugates towards the centre of the platelets is observed after about half-an-hour of platelet–surface interactions.

4.2. Movement of fluorescein-labelled fibrinogen

To confirm the redistribution of fibrinogen–gold conjugates rather than gold particles separated from the conjugates, we examined the redistribution of fibrinogen molecules after labelling with fluorescein. When fluorescein-labelled fibrinogen was precoated on the surface, the fluorescence intensity was homogeneous throughout the surface. When platelets spread on the surface, the distribution of fluorescein-labelled fibrinogen was altered. Fig. 5 shows video images of platelets spread on the surface coated with fluorescein-labelled fibrinogen. The
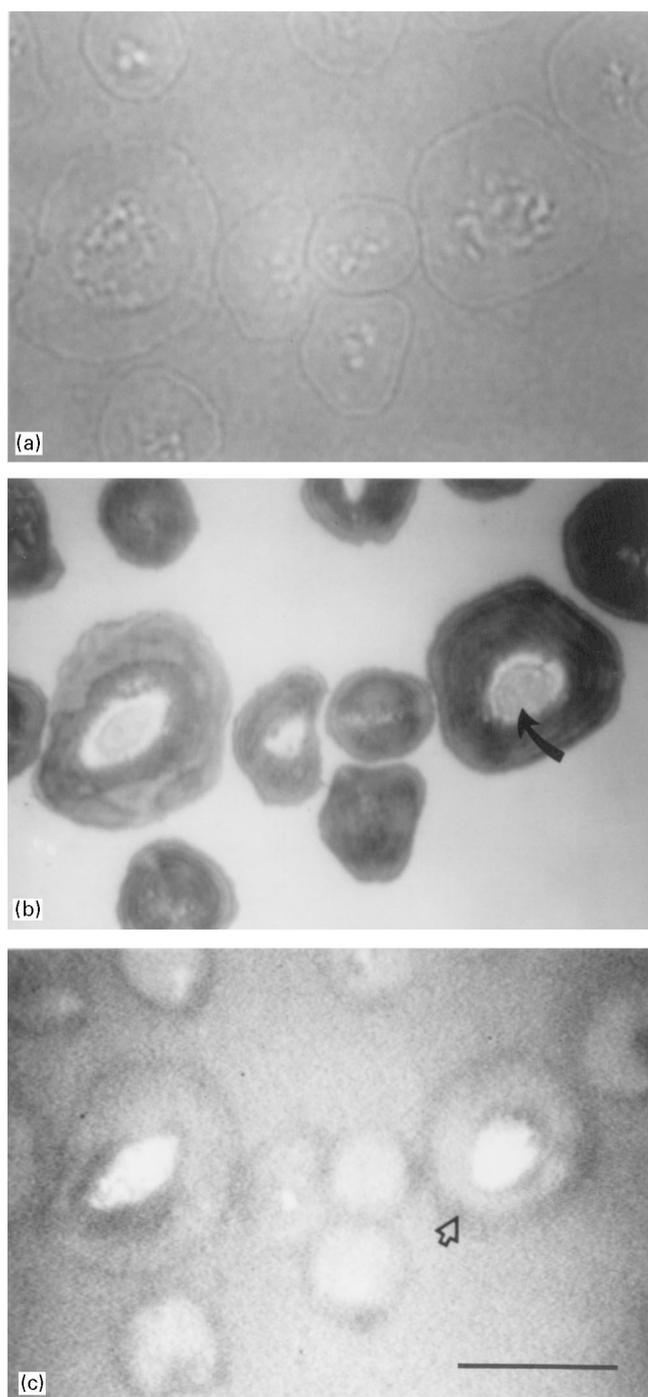
Fig. 5. Images of platelets spread on glass surface coated with fluorescence-labelled fibrinogen observed by: (A) VEBM; (B) VEIRM; and (C) VIFM. In the VIFM image (C) the clustering of fluorescein-labelled fibrinogen at the central granulomere region is clearly seen. The fluoresence intensity at the peripheral web region of spread platelets is lower than the background. The fluorescein-labelled fibrinogen was homogeneously distributed on the surface before exposure to platelets. Fibrinogen was adsorbed to the glass surface at the bulk concentration of 0.1 mg ml^{-1} for 1 h, and then platelets were allowed to spread for 2 h at room temperature.

VEBM image (Fig. 5A) shows granules released from the fully spread platelets. In VEIRM, the centre of the fully spread platelets is white (arrow in Fig. 5B). This indicates that the centre of the spread platelets is separated from the surface by more than 100 nm [5]. The VIFM picture of the same platelets is shown in Fig. 5C. There is a distinct increase in fluorescence intensity in the central granulomere region of the spread platelets. This region of high fluorescence intensity corresponds to the region where fibrinogen–gold conjugates were collected. Figure 5C also shows that the fluorescence intensity of the periphery (white arrow) of spread platelets is lower than that of the background or that of the inner filamentous zone. Clearly, fluorescein-labelled fibrinogen molecules in the peripheral region were picked up by platelets and transferred towards the centre of the spread platelets. Thus, it can be said that the redistribution of gold particles shown in Figs. 1–3 is due to the movement of fibrinogen–gold conjugates, rather than the gold particles themselves.

Figure 6 shows that the redistribution of fibrinogen molecules continues and the centralized fibrinogen molecules are eventually internalized into platelets. The high fluorescence intensity in the centre of the spread platelets disappeared and the central granulomere region appeared dark except for long, narrow areas (arrow in Fig. 6A). The presence of fluorescein-labelled fibrinogen in a narrow space suggests that the fibrinogen molecules were internalized into the open canalicular system (OCS) [26, 27]. As more time was allowed, the intensity of fluorescein-labelled fibrinogen became increasingly smaller and eventually disappeared as indicated by a white arrow in Fig. 6B. It is not likely that the fluorescence signal was bleached, since the background fluorescence signal remained. The disappearance of the fibrinogen collected in the OCS may be due to the collapse or evagination of the OCS as suggested by Leistikow et al. [28]. Thus, it appeared that the fibrinogen molecules on the surface were picked up by fibrinogen receptors on the ventral membrane of platelets, centralized, internalized into the OCS, and somehow removed from the platelets.

5. Discussion

When platelets are activated in suspension, fibrinogen receptors are relocalized to facilitate the role of fibrinogen as a molecular bridge for aggregation of platelets [29]. Estry et al. [27] observed that the binding of soluble, released fibrinogen did not cause the reorganization of fibrinogen receptors. The receptor translocation was induced only by substrate-bound fibrinogen, such as fibrinogen on colloidal gold particles and fibrinogen bound to another platelet. Apparently, the surface-adsorbed fibrinogen caused the receptor translocation. It would be interesting to study the changes that might occur in
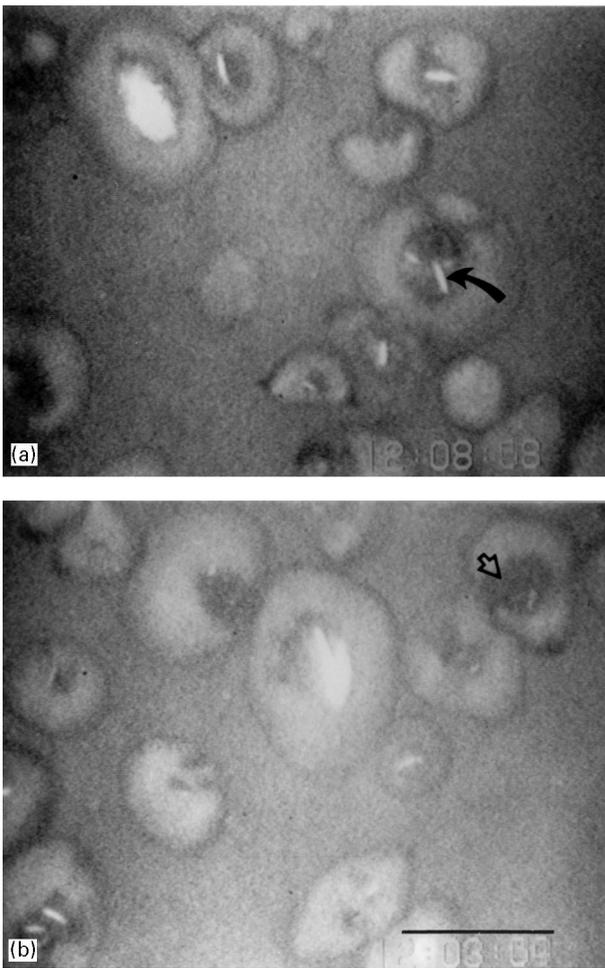
Fig. 6. VIFM images showing the internalization of fluorescein-labelled fibrinogen into the spread platelets. The wide bright area becomes narrow (black arrow in (A)) and eventually becomes black (white arrow in (B). The fluorescence intensity in the black regions becomes lower than the background intensity on the surface. Fibrinogen was adsorbed onto the glass surface at the bulk concentration of 0.1 mg ml^{-1} for 1 h, and then platelets were allowed to spread for 2 h at room temperature.

fibrinogen molecules upon binding to the substrate. The study by Horbett and Lew [30] on the conformational changes of adsorbed fibrinogen examined by antibody binding could provide the extent of conformational changes and its effect on the receptor translocation. The relocalization and movement of the fibrinogen receptors are also well documented on the dorsal membrane of spreading platelets [9, 31]. It is well known now that the binding of colloidal gold-labelled ligand or antibody to the fibrinogen receptor (GPIIb/IIIa) triggers a long-range centripetal movement of the receptor–ligand complexes on the dorsal membrane of spread platelets [32, 33].

In our study we observed that the surface-adsorbed fibrinogen–gold conjugates or fluorescein–labelled fibrinogen molecules move towards the centre of a spreading platelet on the ventral membrane as well as on the dorsal membrane. The movement occurring on the ventral membrane is similar to that previously observed with fibrinogen–gold conjugates on the dorsal membrane of spreading platelets [7, 9]. The redistribution of the surface-adsorbed fibrinogen by spreading platelets was also observed by Gaebel and Feuerstein [34]. In a study using a fluorescent probe-labelled fibrinogen, they noticed a dark outer ring with an inner region having fluorescent areas, with greater intensity than regions not covered by platelets. In subsequent studies, Feuerstein and Sheppard [35] suggested that the shrinking of adherent platelets caused areas free of pre-adsorbed fluorescence probe-labelled fibrinogen to be exposed. We have observed that when platelets spread they undergo continuous expansion and shrinking at the peripheral web region and move in different directions, while the centre of the platelet remains at the same position. As a result each platelet covers more space than the final size of the spread platelet. This may appear to be shrinking of the platelets. It is noted, however, that the fibrinogen-free region in Fig. 5 is still under the spread platelets. The removal of the surface-adsorbed fibrinogen in that region is most likely to be due to the centripetal movement of fibrinogen receptors on the ventral membrane of platelets.

Fibrinogen–gold conjugates bind diffusely to spread platelets, but are translocated rapidly from the peripheral margin to cell centres and to channels of the OCS. The internalization of fibrinogen molecules into the OCS has been observed by many investigators [8, 26, 28, 36, 37]. Initial binding of fibrinogen–gold conjugates to the surface of ADP-stimulated platelet in suspension was immediately followed by gold accumulation in plasmalemma pits subjected to further internalization [37]. After prolonged (20 min) incubation, fibrinogen–gold conjugates were localized in vacuole-like and/or granule-like structures. The surface-connected OCS is known to be a final common pathway for uptake of particulates (i.e. transport of substances into the cells) and discharge of secretary products in activated platelets [38]. The two functions are known to take place simultaneously with different speed limits [26]. The translocation of surface-adsorbed fibrinogen–gold conjugates and fluorescein-labelled fibrinogen to the centre of spreading platelets and to the OCS was also observed on the ventral membrane. As shown in Figs. 5 and 6, fibrinogen molecules on the substrate surface appear to be taken up into the OCS and eventually removed from the platelets. At this point it is not clear whether the fibrinogen molecules were degraded or not, but the process indicates that there is a mechanism to recycle the fibrinogen molecules. It appears that exogenous fibrinogen, after initial binding to its receptors, may play a role not only in aggregation but also in activation-related cellular responses [37].

As shown in Fig. 4, the fibrinogen–gold conjugates moved with a velocity ranging from less than 0.1 μm min^{-1} to more than 10 μm min^{-1} depending on

the location on the ventral membrane. A majority of the fibrinogen–gold conjugates for the velocity measurements were observed on the filamentous zone of spread platelets. When the pathway of a fibrinogen–gold conjugate in the peripheral web region was traced, the reduction in the velocity of the conjugate between the web and outer filamentous zone was apparent. When the conjugates reached the inner filamentous zone, they sometimes hesitated before continuing to move. In some cases, the conjugates changed direction to either right or left before continuing centripetal movement. Some fibrinogen–gold conjugates in the inner and outer filamentous regions disappeared from the screen for a while and appeared again at nearby positions while maintaining general centripetal movement.

The significance of the centralization of fibrinogen receptors on the ventral membrane of spreading platelets has not been fully understood. In suspension, the clustering and internalization of 'occupied' membrane receptors may also contribute to the formation of close contacts between platelets stimulated by primary agonists in the presence of exogenous fibrinogen and other adhesive proteins [37]. The centripetal movement of fibrinogen–gold conjugates could be a simple clearance mechanism or a part of the complex communication process which transfers information inside the platelets. It could also be related to the platelet removal from the surface, i.e. an embolization process. For example, the removal of the surface fibrinogen may be related to breaking of the adhesion between platelets and the surface. The clearing of the surface-adsorbed fibrinogen by spreading platelets also suggests that the capacity of the surface to activate platelets is reduced once the surface is exposed to platelets. When biomaterials are exposed to blood, the biomaterial surface activates platelets and coagulation factors, which leads to mural thrombus formation [39]. Within hours, however, the surface loses much of its capacity to activate platelets and the surface becomes passivated. This short-term surface passivation has not been fully explained. The removal of the surface-adsorbed fibrinogen by spreading platelets may explain this at least partially. The fully spread platelets, which form the basis for the thrombus formation, may remove fibrinogen from the surface. More studies under various conditions are necessary to understand the significance of the redistribution of the surface-adsorbed fibrinogen molecules by spreading platelets. It may in turn provide more insights into the long-term platelet–surface interactions, and thus the development of non-thrombogenic biomaterials.

Acknowledgements

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