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The solubilization and morphological change of human platelets in various detergents

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The solubilization of human gel-filtered platelets by octyl glucoside, Triton X-100, dodecylsulfate, and deoxycholate was compared from the analysis of (1) cell lysis, (2) marker leakiness, and (3) component solubility. These analyses all revealed that the effect of detergent concentration on the solubilization of platelets by these detergents was exerted in three stages, i.e., the prelytic, lytic, and complete platelet-lysis stages. These analyses also indicated several differences among platelets in these detergents. (i) The ratio of the platelet-saturation concentration (PSC) to critical micellar concentration (CMC) was about 1/2 for octyl glucoside, Triton X-100 and dodecylsulfate, while it was close to 1 for deoxycholate. (ii) Platelets in octyl glucoside, Triton X-100, and dodecylsulfate all showed parallel curves in cell lysis, protein solubilization and marker leakiness, while the platelet lysis in deoxycholate was identical to the phospholipid solubilization. (iii) The solubility curves of various components in Triton X-100 and deoxycholate were parallel. However, the solubility of cholesterol in octyl glucoside was lower than that of protein and phospholipid. In dodecylsulfate, the solubility of phospholipid and cholesterol was very low in comparison with that of protein. In addition, morphological studies using scanning electron microscopy (scanning EM) revealed that the solubilization by octyl glucoside or Triton X-100 might occur via membrane area expansion. On the other hand, the solubilization by dodecylsulfate or deoxycholate showed membrane vesiculation prior to cell lysis. Moreover, in the prelytic stage, the morphological change in platelets in octyl glucoside showed only concentration dependence by swelling to an ellipsoid and then to a sphere. However, the morphological change in platelets in the other three detergents was dependent not only on the detergent concentration but also on prolonged incubation. Specifically, in Triton X-100, the cells initially changed to spiculate discs and then reached their final shape as swollen discs with surface invagination. In dodecylsulfate and deoxycholate the morphological changes were almost the same. The cell initially deformed in shape to a spiculate disc and finally to a stretched-out flat form. The results are discussed according to the bilayer couple hypothesis. Also, in the prelytic stage, these detergents caused inhibition of the response of platelets to collagen and ADP-fibrinogen.

Introduction

Detergents are useful for the extraction of membrane components and the reconstitution of proteins into a defined liposome [1–7]. The choice of a suitable detergent is usually based on its ability to preserve enzymatic activity. However, detergents are amphipathic in nature and can infiltrate into the membrane bilayer to interact with enzymes in the hydrophobic domain. The ionic charge or nonionic nature of detergent, as well as the critical micellar concentration (CMC) are among the factors that can modify the detergent–enzyme interactions [8–11]. Hence, these factors have to be considered during membrane solubilization.

The solubilization of a membrane by a detergent is based on the conversion membrane bilayer to mixed micelles of detergent and membrane constituents. Depending on the detergent concentration, the solubilization appears to occur in three stages [8,9]. The prelytic

Abbreviations: scanning EM, scanning electron microscopy; CMC, critical micellar concentration; PSC, platelet-saturation concentration; MCPLC, minimum complete platelet-lysis concentration; S_{50}, the detergent concentration required to solubilize 50% of an indicated component.

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stage occurs at the concentration of the detergent which enables it to enter into the membrane without rupturing the bilayer structure. The lytic stage begins once the membrane saturation concentration has been reached. When all membrane constituents become mixed micelles, this marks the onset of complete cell-solubilization stage. It is conceivable that each stage of membrane solubilization by detergents will influence the functional activity of the enzyme system.

Based on lyotropic mesomorphism, the mode of detergent insertion has been classified into two types [8]. A type A detergent inserts into the membrane in its monomeric form. The hydrophobic moiety of the molecule is linear, such as is the case with octyl glucoside, Triton X-100 and dodecylsulfate. A type B detergent is incorporated in the membrane in an oligomeric form. The hydrophobic moiety of such a molecule, e.g., deoxycholate, is complicated. The type A detergents may cause membrane lysis by increasing bilayer curvature [12], while the type B detergents may chop up the bilayer into small disc-like pieces [8]. In addition, these detergents behave differently in the solubilization of membrane components [13–16].

Membrane-active agents can cause changes in platelet membrane morphology, which include both the lipid bilayer and the membrane skeleton [17–20]. The platelets in chloropromazine and lysophosphatidylcholine change morphologically to a sphere and spiculate sphere, respectively. The bilayer couple hypothesis explains that this differential change in morphology is due to the different asymmetric distribution of these two solutes between the bilayer leaflets [20]. Besides the membrane morphology, the platelet shape change also results from the reorganization of cytoplasmic actin filament and microtubule coil [21–27].

This study has analyzed the solubilization of human platelets by four kinds of detergent which are widely used in membrane solubilization [4–7]. The physical properties of these detergents are well characterized [8–11]. Dodecylsulfate is an ionic detergent (type A). Octyl glucoside and Triton X-100 are both nonionic detergents (type A), which have considerably different CMCs. Deoxycholate is a bile salt (type B). The solubilization was investigated by analyzing the cell lysis, marker leakiness, and solubility of membrane components. The results indicated that the solubilization of platelets by these detergents occurs in three stages. The platelet-saturation concentration (PSC) and the minimum complete platelet-lysis concentration (MCPLC) were estimated. The former is the detergent concentration at which the conversion of the membrane to mixed micelles begins. The latter is the minimum detergent concentration required for all of membrane components to become mixed micelles. In the prelytic stage, further studies were performed to measure the effect on cell morphology as revealed by scanning EM and by the agonist-stimulated response. These results showed that platelets in various detergents exhibited different component solubilization and morphological change. These detergents also inhibited the response of platelets to collagen and ADP-fibrinogen.

Experimental Procedures

Materials

Chemicals, organic solvents, Triton X-100, sodium dodecylsulfate, sodium deoxycholate, bis(trimethylsilyl) trifluoroacetamide, EDTA (Titriplex II) and thin-layer chromatographic plate (TLC plate silica gel 60) were purchased from E. Merck (Darmstadt, F.R.G.). All organic solvents were redistilled before use. The following reagents were obtained from Sigma (St. Louis, MO, U.S.A.): octyl glucoside, bovine thrombin, collagen (type II from calf skin), adenosine diphosphate (platelet aggregation agent), fibrinogen (from man), phorbol 12-myristate 13-acetate, NADH, quin2 acetoxymethyl ester (quin2/AM) and fatty acid-free bovine serum albumin. Arachidonic acid was from Boehringer-Mannheim (Mannheim, F.R.G.), platelet-activating factor was from Serva (Heidelberg, F.R.G.) and Sepharose 2B was from Pharmacia (Piscataway, NJ, U.S.A.).

Methods

Preparation of gel-filtered platelets. The preparation of gel-filtered human platelets has been detailed previously [24,27]. It was carried out on this occasion according to the method of Lages et al. [28]. Human blood was freshly drawn from healthy donors and anticoagulated with 10% (v/v) of 0.11 M sodium citrate. Unless otherwise specified, the buffer solution was a modified calcium-free Tyrode's buffer containing 0.1% dextrose and 0.2% of bovine serum albumin [28]. The cell number was estimated in a hemacytometer by a phase contrast microscope (Nikon, Type 104, Tokyo, Japan). The isolated platelets were preincubated at 37 °C for 30 min prior to further studies [27].

Analyses of platelet lysis. The percentage of cell lysis was estimated from the change of optical turbidity in an aggrecorder (Hitachi, Model PA-2310, Kyoto, Japan). The optical absorbance of $3 \cdot 10^8$ cell/ml was 0.5. A time-course of 50 min was followed.

Estimation of the marker leakiness. Platelets ($3 \cdot 10^8$ cells/ml) were incubated the detergents at various concentrations. After a certain period, aliquots were introduced with 2.5 mM EDTA and then centrifuged in an laboratory centrifuge (Sigma Model 202 CM) at 13500 $\times g$ for 1 min. The supernatant was taken to analyze the content of various cellular markers. The activity of lactate dehydrogenase (cytosolic marker) was estimated according to the method of Bergmeyer and Bernt [29], and that of acid hydrolase (lysosomal marker) was

measured by following the method of Dangelmaier and Holmsen [30]. The substrate of the latter enzyme was *p*-nitrophenyl-*N*-acetyl β-D-glucosaminide. The release of serotonin (dense-granule marker) was assayed as that described by Drummond and Gordon [31]. These detergents and EDTA did not affect these markers. Hence, 100% of each marker was the value estimated in the supernatant of platelets treated with 0.1% Triton X-100.

Estimation of the component solubilization. In various detergents, the studies on the time-course of the component solubilization were performed as described above. For estimating the protein solubility, only the protein content in the pellet was measured. The pellet was washed three times with the Tyrode's buffer plus 2.5 mM EDTA, before the quantitation. The protein content was determined according to the method of Lowry et al. [32]. The total protein content in the pellet of a parallel control experiment was taken as 100%. For analyzing the lipid solubilities, the obtained supernatant and pellet were both quantitated to obtain the percentage of lipid solubility. Lipids were extracted according to the method of Bligh and Dyer [33]. Phospholipid composition was analyzed by two-dimensional thin-layer chromatography as described previously [24], and quantitated by estimating the phosphorus content as described previously [34,35]. Cholesterol content was estimated by gas chromatography (Shimadzu, GC-9A, Japan) as described by Derks et al. [36]. The derivative lipids of bis(trimethylsilyl)trifluoroacetamide were separated at 240°C with a flow rate of 40 ml/min. The column (3 × 2 mm) was packed with 10% SP 2330 on chromosorb WAW-DMCS 80/100 (Supelco, Bellefonte, PA).

Morphological studies by electron microscopy. The sample preparation has been detailed in previous work [27]. In brief, the detergent-treated platelets were added with 5 vol. of ice-cold 2.5% glutaraldehyde in modified calcium-free Tyrode's buffer (pH 7.4). After a storage in ice for 1 h, the prefixed samples were postfixed with 1% OsO_4 and dehydrated. For the scanning EM study, the samples were dried in a critical-point dryer (Hitachi HCP-2, Japan) in CO_2, and then plated with gold in an ion coater (Eiko Engineering, Model IB-2). The plated samples were studied under a Hitachi S-520 scanning electron microscope at 20 kV.

Other analyses. Commercially available quin2/AM was employed to measure the cytosolic free Ca^{2+} concentration of platelets in various prelytic detergent concentrations. The procedure was modified according to the method of Tsien et al. [27,37].

The study of agonist-stimulated response was performed in an aggrecorder (Hitachi, Model PA-3210, Kyoto, Japan), studying agonist-stimulated aggregation. The agonists were thrombin (0.1 unit/ml), collagen (100 μg/ml), ADP (20 μM) plus fibrinogen (100 μg/ml), arachidonic acid (100 μM), phorbol 12-myristate 13-acetate (10 ng/ml), and platelet-activating factor (100 ng/ml).

Results

Solubilization of human platelets by octyl glucoside, Triton X-100, dodecylsulfate and deoxycholate

Fig. 1 depicts the solubilization of gel-filtered platelets by various concentrations of octyl glucoside, Triton X-100, dodecylsulfate and deoxycholate. The solubilization was investigated by estimating the percentage of (1) cell lysis, (2) marker leakiness, and (3) solubility of protein, phospholipid and cholesterol. The markers were lactate dehydrogenase, acid hydrolase and serotonin. Serotonin leakiness in each kind of detergent was identical to acid hydrolase leakiness (data not shown). These three analyses of the effect of detergent concentration showed the solubilization curves of platelets in each detergent to be comparable. The sigmoidal curves indicated the solubilization to occur in three stages, i.e., prelytic, lytic and complete platelet-lysis. At the various concentrations producing the lytic stage, the solubilization of platelets by the same detergent required the same incubation period to reach plateau, but the incubation period was different among these detergents. Hence, the solubilization data in each detergent were obtained from different incubation periods, i.e., 10 min in octyl glucoside; 30 min in Triton X-100; 30 min in dodecylsulfate; and 20 min in deoxycholate (Table I).

Two physical parameters, PSC and MCPLC, were estimated from the intersections of extrapolated linear portions of the sigmoidal cell-lysis curves. These data are summarized in Table I. The PSC of each detergent might reflect its membrane saturation concentration in the platelet. The ratios of PSC/CMC of octyl glucoside, Triton X-100 and dodecylsulfate were all about 1/2, but that of deoxycholate was close to 1. The MCPLC values of octyl glucoside, Triton X-100 and dodecylsulfate were similar to their own CMC, while that of deoxycholate was double its CMC. By comparing the ratio of MCPLC/PSC, it was shown that the ratio was 4 for octyl glucoside and 2 for the other three detergents. Hence, to complete the platelet solubilization, more octyl glucoside might be required than the other three detergents.

In all of these detergents, the marker-leakiness study indicated that more than 90% of the platelet population was intact in the prelytic stage. Interestingly, the cytosolic free Ca^{2+} concentration of the treated platelet was similar to the control (data not shown). However, in the lytic stage, several different findings were observed among platelets in these detergents by comparing the detergent-concentration profiles of these three analyses (Fig. 1). (i) Platelets in each kind of detergent showed identical profiles in protein solubilization, leakiness of lactate dehydrogenase and acid hydrolase. Also the

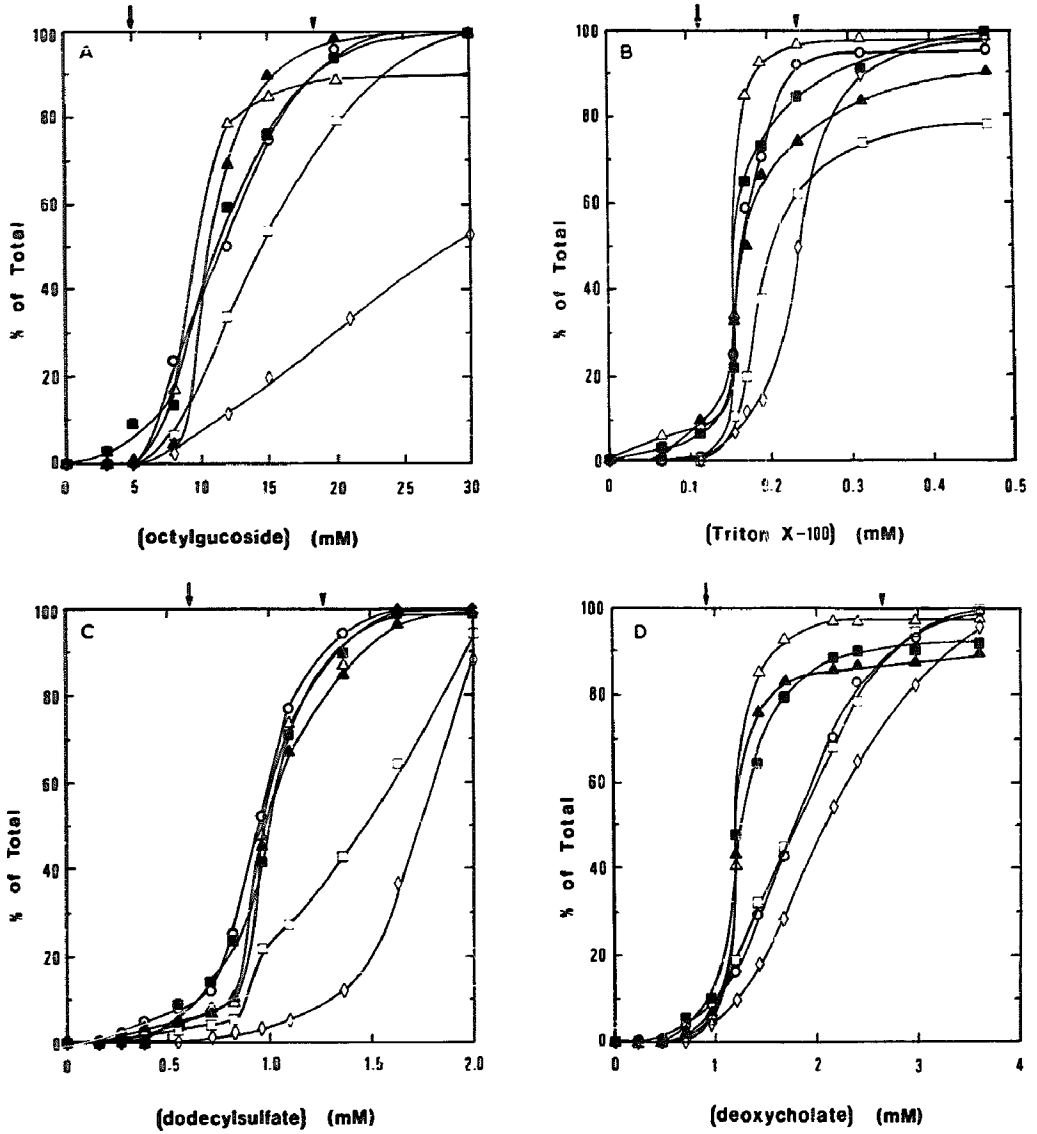

Fig. 1. Solubilization of human platelets by detergents. Gel-filtered platelets ($3 \cdot 10^8$ platelets/ml) were incubated with various concentrations of (A) octylglucoside for 10 min, (B) Triton X-100 for 30 min, (C) dodecylsulfate for 30 min, and (D) deoxycholate for 20 min. Experimental procedures detail the estimations of the percentage of cell lysis (○), the leakiness of lactate dehydrogenase (△) and acid hydrolase (▲), and the solubilities of phospholipid (□), protein (■) and cholesterol (◇). Data points were taken from an average of at least six separate experiments. Arrows and arrow heads at the top of the figures represent the PSC and MCPLC, respectively. The data were determined from the itersections of the extrapolated linear portions of the sigmoidal cell-lysis curve.

composition of phospholipid species in the solubilized fraction was similar to that in whole cell (data not shown). Hence, the percentage of cell lysis in various lytic concentrations might represent the gradual lysis of the different platelet population. (ii) The cell-lysis profile in octyl glucoside; Triton X-100, or dodecylsulfate was parallel to that of membrane leakiness studies (Fig. 1A–C), while the profile in deoxycholate was identical to that of phospholipid solubilization (Fig. 1D). This may mean that only deoxycholate caused membrane leakiness prior to cell lysis. (iii) The solubility of membrane components in these detergents was in the order

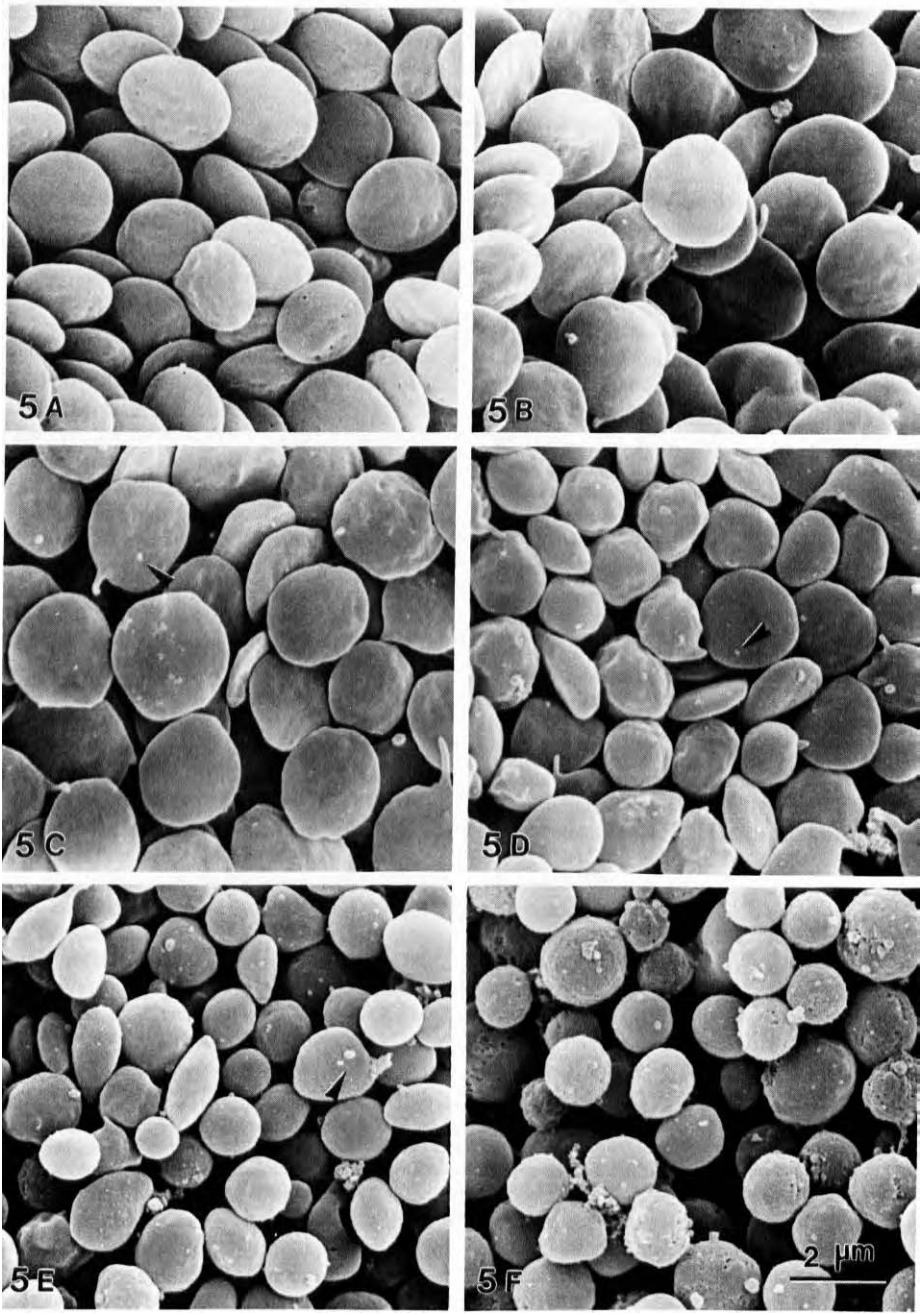

Fig. 2. Scanning electron micrographs of gel-filtered platelets in octyl glucoside at various concentrations (×8000). The gel-filtered platelets were preincubated at 37 °C for 30 min (A), and then incubated for another 10 min with octyl glucoside in various prelytic concentrations of 1 mM (B), 2 mM (C) and 5 mM (D), and in various lytic concentrations of 8 mM (E) and 11 mM (F). Arrow head indicates the bleb on the platelet surface. Details of experiments are given in Experimental procedures.

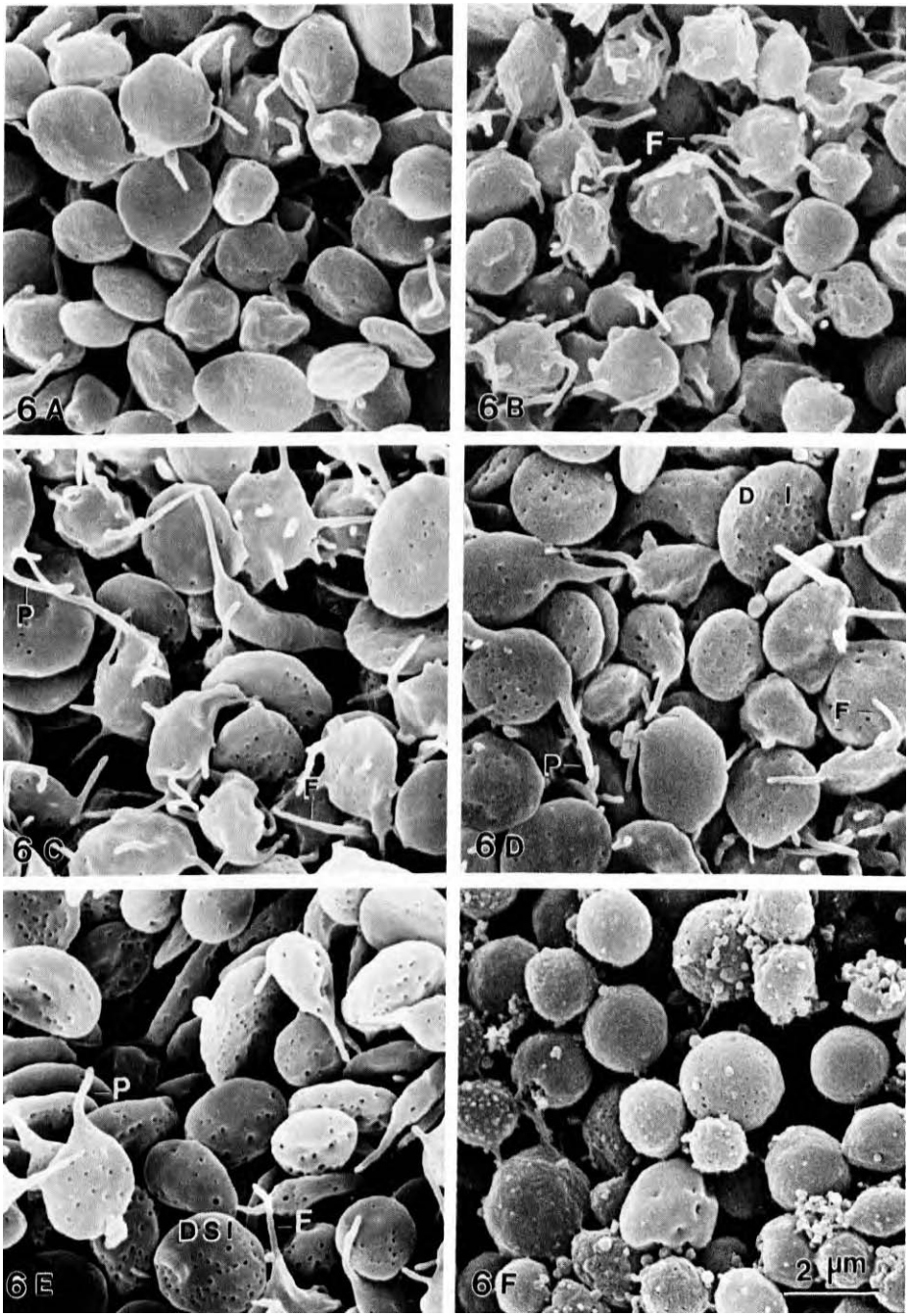

Fig. 3. Scanning electron micrographs of gel-filtered platelets in Triton X-100 at various concentrations (×8000). The prewarmed platelets (Fig. 2A) were added with Triton X-100 in a prelytic concentration of 0.02 mM for 10 min (A), and in another prelytic concentration of 0.08 mM for different periods of 10 (B), 20 (C), 30 (D) and 40 (E) min. Also, the cells were incubated in a lytic concentration of 0.16 mM for 5 min (F). The abbreviations in parentheses indicate the filopodium (F) (width < 200 nm), the pseudopodium (P) (width > 200 nm) and the discoid cells with surface invagination (DSI) (hole is about 100 nm in diameter). Details of experiments are given in Experimental procedures.

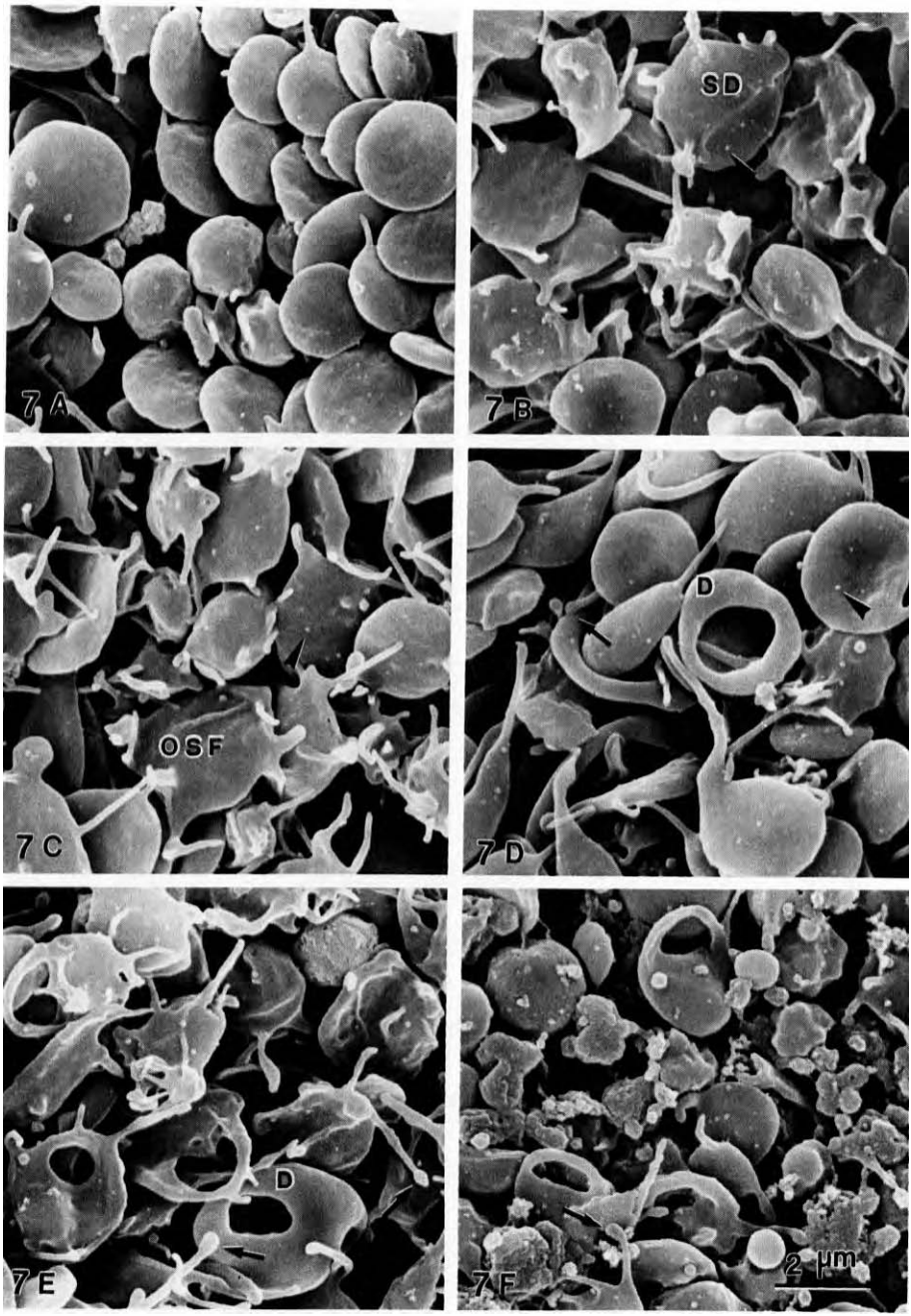

Fig. 4. Scanning electron micrographs of gel-filtered platelets incubated in dodecylsulfate at various concentrations (×8000). The prewarmed platelets (Fig. 2A) were added with dodecylsulfate in a prelytic concentration of 0.20 mM for 10 min (A), and in another prelytic concentration of 0.65 mM for different periods of 10 (B), 30 min (C) and 50 (D) min. Also, the cells were in a lytic concentration of 1.3 mM for different periods of 5 min (E), and 10 min (F). The abbreviations in parentheses indicate the spiculate discoid cell (SD), stretched-out flat cell (OSF) and doughnut-shape cell (D). The arrow head indicates the bleb on the platelet surface, while the arrow indicates the filopodium with enlarged tip on the surviving cell. Details of experiments are given in Experimental procedures.

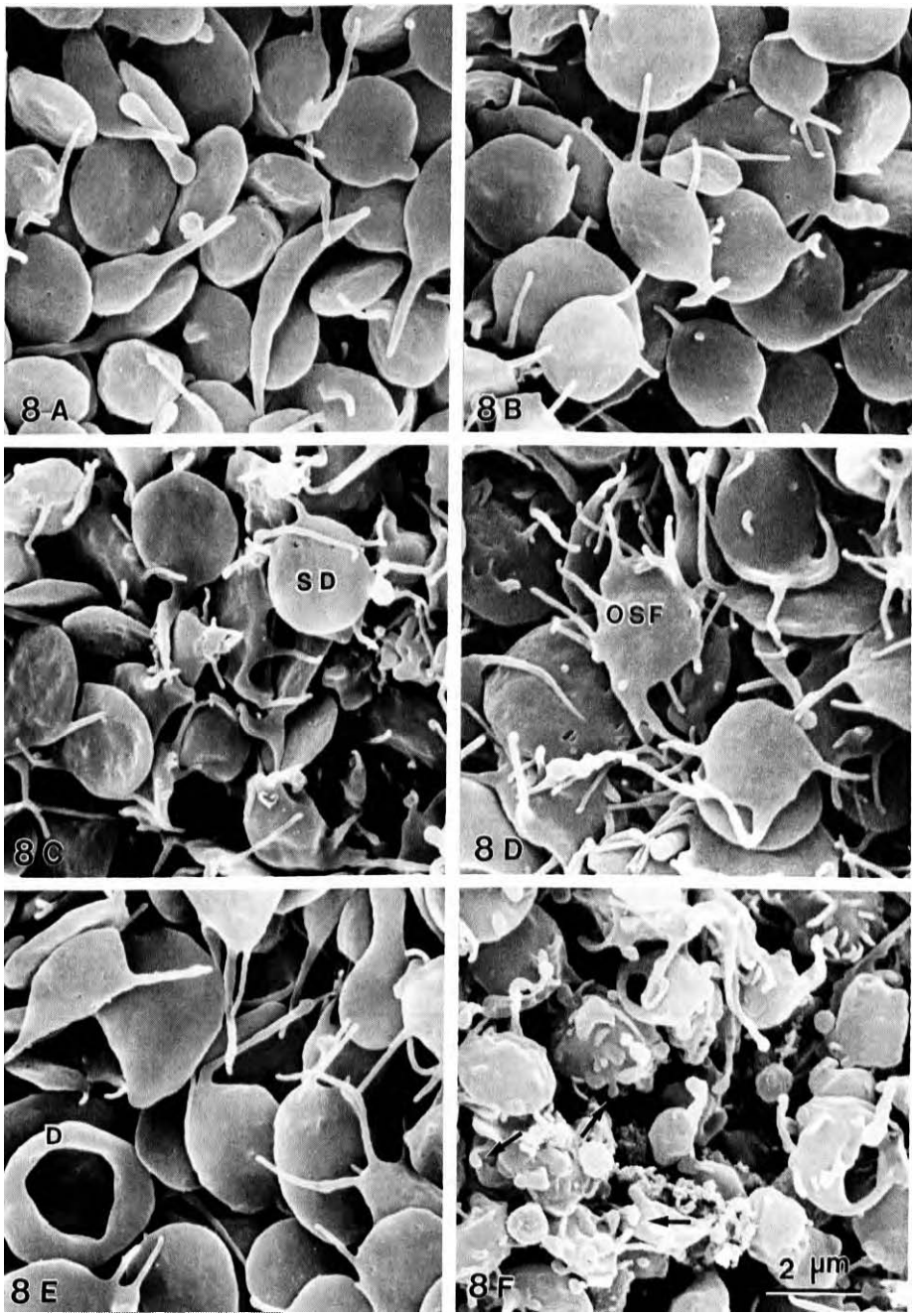

Fig. 5. Scanning electron micrographs of gel-filtered platelets incubated in deoxycholate at various concentrations (×8000). The prewarmed platelets (Fig. 2A) were added with deoxycholate in various prelytic concentrations of 0.12 mM (A), and 0.48 mM (B) for 10 min, and in a prelytic concentration of 0.96 mM for different periods of 10 (C), 20 (D) and 50 (E) min. Also, the cells were in a lytic concentration of 1.68 mM for 5 min (F). The abbreviations in parentheses indicate the spiculate discoid cell (SD), stretched-out flat cell (OSF) and doughnut-shape cell (D). The arrow indicates a filopodium with enlarged tip on a surviving cell. Details of experiments are given in Experimental procedures.

protein > phospholipid > cholesterol. Table I tabulates the estimated S_{50}, the concentration of a detergent required to solubilize 50% of each component indicated. In octyl glucoside, the solubility curve of phospholipid was parallel to that of protein, while the solubility of cholesterol was poor (Fig. 1A). In Triton X-100 and deoxycholate, the solubility curves of phospholipid and cholesterol were parallel to that of protein (Fig. 1B, D).

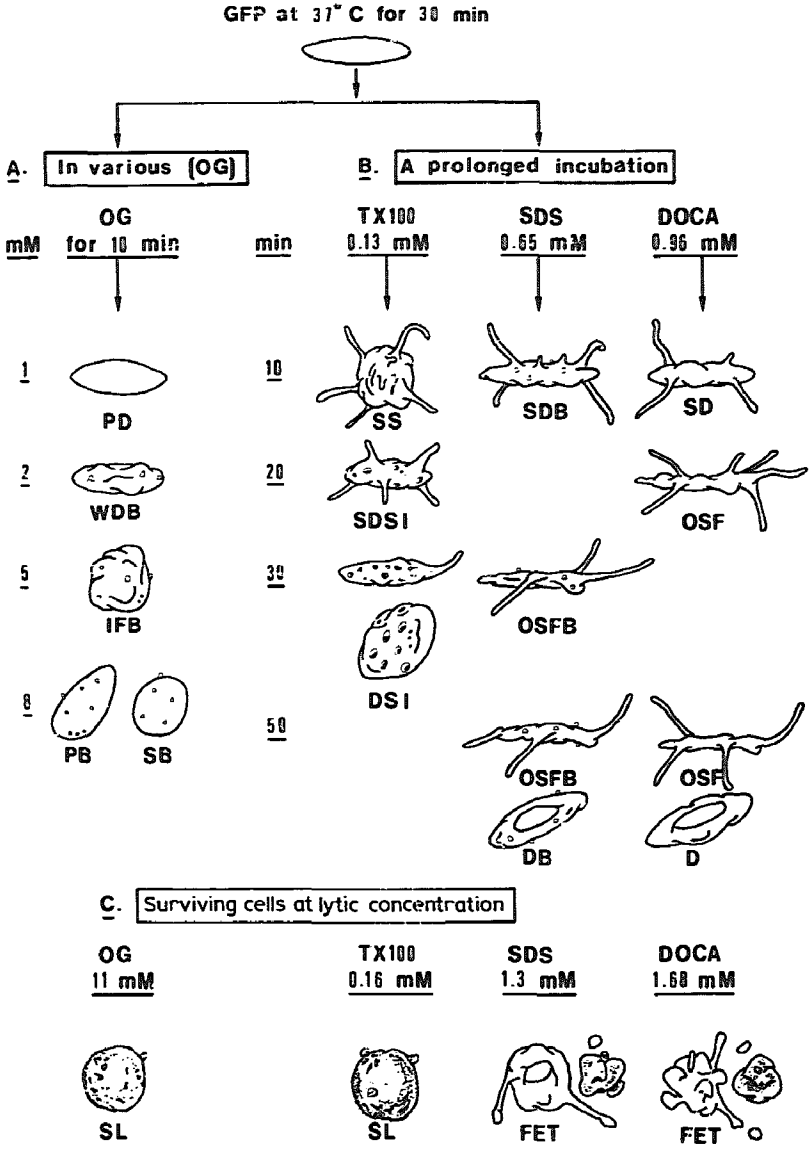

Fig. 6. Schematic drawings showing the changes in platelet morphology at various octyl glucoside concentrations (A); in a prolonged incubation at platelet saturation concentration of Triton X-100, dodecylsulfate and deoxycholate (B); and at the lytic detergent concentration (C). Abbreviations: GFP, gel-filtered platelet; OG, octyl glucoside; TX100, Triton X-100; SDS, dodecylsulfate; DOCA, deoxycholate; PD, puffy disc shape; WDB, wavy disc shape with blebs; IFB, irregular form with blebs; PB, pear shape with blebs; SB, sphere with blebs; SS, spiculate sphere; SDSI, spiculate disc with surface invagination; DSI, disc with surface invagination; SDB, spiculate disc with blebs; SD, spiculate disc; OSFB, stretched-out flat shape with blebs; OSF, stretched-out flat shape; DB, doughnut shape with blebs; D, doughnut shape; SL, spongy-lysis form; FET, surviving cell showing filopodium with enlarged tip.

TABLE I

The physical properties of various detergents in the solubilization of human platelet

Physical property	Nonionic detergent		Anionic detergent	
	octyl glucoside	Triton X-100	dodecyl-sulfate	deoxy-cholate
PSC [a] (mM)	5.00	0.13	0.65	0.97
CMC [b] (mM)	14.5-25	0.24	1.33	0.91
PSC/CMC	0.35	0.55	0.50	1.07
Lysis time [c] (min)	< 10	30	> 30	20
MCPLC [a] (mM)	18.0	0.23	1.21	2.60
MCPLC/PSC	3.60	1.75	1.86	2.68
S_{50} [d] (mM)				
Protein	11.0	0.16	0.96	1.20
Phospholipid	14.4	0.20	1.45	1.84
Cholesterol	20.5	0.24	1.74	2.08

[a] PSC (platelet-saturation concentration) and MCPLC (minimum complete platelet-lysis concentration) were determined from the extrapolation of the cell lysis data in Fig. 1.

[b] CMC (critical micellar concentration) was taken from the data of previous works [8–11].

[c] Lysis time represents the required incubation period for the complete solubilization of platelets in various lytic concentrations.

[d] S_{50} is the detergent concentration required to solubilize 50% of each component indicated.

In dodecylsulfate, the solubilities of phospholipid and cholesterol appeared to be very low as compared with that of protein (Fig. 1C). In summary, the results indicate differential solubilization of platelets by detergents of different physical properties.

Morphological change of platelets in octyl glucoside, Triton X-100, dodecylsulfate and deoxycholate

Scanning EM was employed to investigate how platelet morphology was affected by these detergents (Figs. 2–5). In various prelytic concentrations, platelets in octyl glucoside changed morphology only in a concentration-dependent fashion, while in the other three detergents, the changes depended on both detergent concentration and incubation period. A sequential morphological change appeared in a prolonged incubation. These changes are depicted by a schematic drawing in Fig. 6.

In octyl glucoside, platelets changed to spheres, which remained the same throughout the incubation from 30 s to 50 min. This mean that the uptake of octyl glucoside reached equilibrium within seconds (Fig. 2). In 1 mM of octyl glucoside platelets changed shape to ellipsoids with tiny blebs on the cell surface (Fig. 2B). Increasing the octylglucoside concentration, the cells gradually swelled to the spherical shape with an increase in the number of blebs (Figs. 2C–E). At the lytic concentration of 11 mM, pellet of the surviving platelets had a spongy spherical form (Fig. 2F). Hence, cell lysis might have occurred via membrane-area expansion.

Interestingly, platelets in Triton X-100 changed their morphology in a time-dependent fashion (Fig. 3). After the addition of Triton X-100, the cells immediately either changed to spiculate spheres or acquired a tadpole-like shape (Fig. 3A, B). After a prolonged incubation, the deformed cells gradually attained their final form as swollen discs showing a number of surface invaginations (about 100 nm in diameter). Also, the number of filopodia decreased (Fig. 3C). It took 30 min for the platelets to go through this sequential morphological change (Figs. 3D, E). This may mean a prolonged incubation of 30 min was necessary for Triton X-100 to reach equilibrium in the platelet membrane. The cell lysis by Triton X-100 might also occur via membrane area expansion, since at the lytic concentration of 0.16 mM, the remaining pellet of surviving platelets had a spongy-spherical shape (Fig. 3F).

In both anionic detergents, platelets change in morphology to two cell types (Figs. 4, 5). The first type is the spiculate disc, and the second type is the stretched-out flat shape (Figs. 4A, 4B, 5A–C). The population distribution of these two cell types was both dose- and time-course-dependent. The spiculate discoid cells (first type) were more numerous at lower concentration or after a shorter incubation period, while the stretched-out flat cells (second type) were more common at a higher concentration or after a prolonged incubation. Also, after a prolonged incubation, the rim of the second-type cell was more extended (Figs. 4B–D, 5C–E). At 50 min incubation, 10% of the population acquired a doughnut shape, indicating that the membrane fusion occurred from the cytosolic side (Figs. 4D, 5E). Two differences were found in the morphological changes of platelets in dodecylsulfate and deoxycholate. One was that some tiny blebs appeared on the surface of the deformed cells in dodecylsulfate, but no bleb was found on the surface of platelets in deoxycholate. The other was that a longer incubation period was necessary for platelets in dodecylsulfate to change to the stretched-out flat cell type (30 min), while platelets in deoxycholate required 20 min. These two detergents might cause membrane vesiculation before cell lysis. Since at the lytic concentration the surviving cells displayed a mixed population of small vesicles, irregular spongy lysis cells, and deformed cells showing filopodia with enlarged tip (Figs. 4E, 4F and 5F).

In summary, the results indicate that the detergents had different effects on platelet morphology. Also, nonionic detergents might cause cell lysis by membrane area expansion, while anionic detergents caused vesiculation prior to cell lysis.

Effect on the agonist-stimulated response by octyl glucoside; Triton X-100, dodecylsulfate and deoxycholate

Since these detergents caused changes in platelet morphology, the effect of detergent on the response of

TABLE II

Effects of various detergents on platelet aggregation

Data (mean ± S.D.) were taken from at least six experiments in which platelets were preincubated in each detergent for 30 min before the addition of agonist. They are expressed as % of control, where 100% of control equals the cell aggregation of gel-filtered platelets stimulated by each kind of agonist. Details of experiments are given in Experimental procedures. Each indicated detergent concentration was its PSC (platelet-saturation concentration).

Agonists	Nonionic detergent		Anionic detergent	
	octyl glucoside (5 mM)	Triton X-100 (0.12 mM)	dodecyl-sulfate (0.65 mM)	deoxy-cholate (0.96 mM)
Collagen	1.0 ± 1.0	5.6 ± 1.4	49.0 ± 2.0	2.0 ± 2.0
ADP plus fibrinogen	2.0 ± 1.8	25.2 ± 5.5	27.0 ± 6.7	47.8 ± 3.4
Thrombin	92.0 ± 6.0	88.0 ± 5.0	99.0 ± 2.5	97.8 ± 4.6
Phorbol ester	91.8 ± 3.8	57.0 ± 2.5	96.9 ± 4.5	98.4 ± 3.8

platelets to agonist stimulation was investigated and is summarized in Table II. The concentration of each detergent used in this study was its PSC. In these detergents, none of the gel-filtered platelets showed any aggregation, even after a prolonged incubation of 50 min. In each kind of detergent, the cells displayed inhibited responses to collagen and ADP-fibrinogen, but not to thrombin or phorbol ester. Only at the PSC of Triton X-100 was the response to phorbol ester partially inhibited. In addition, we found that 100 μM of arachidonic acid and 100 ng/ml of platelet-activating factor might enhance platelet lysis in these detergents. A prolonged incubation resulted in an increase in the extent of inhibition of the response of platelets to collagen, but not of the response to ADP-fibrinogen (data not shown). Studies are being carried out in this laboratory on the effect of detergent currently on the biochemical events in platelets.

Discussion

This study compares the solubilization of human platelets by octyl glucoside, Triton X-100, dodecylsulfate and deoxycholate. Platelets in these four detergents all exhibit the three-stage solubilization. However, the component solubility and morphological change of platelet varies in these detergents (Figs. 1–5).

The three-stage solubilization of platelets indicates that the onset of cell lysis occurs after the saturation of detergent in platelet membrane (Fig. 1). The physical property of a detergent may determine its insertion into platelet membrane, since the order of PSC and that of CMC are comparable among these detergents (Table I; Refs. 8–11). The estimated PSC/CMC ratios of octyl glucoside, Triton X-100 and dodecylsulfate are similar, and equal to 1/2 of that of deoxycholate. These results are comparable with those found in liposomes [13], human erythrocytes [14], sarcoplasmic reticulum [15] and hepatic microsomes [16]. Hence, the mode of detergent insertion in platelet membrane may be in monomeric form for octyl glucoside, Triton X-100 and dodecylsulfate (type A), and dimeric form for deoxycholate (type B) [8].

The solubilization by these detergents may be an all-or-none process, since platelets in the lytic stage of detergent action show that: (1) the percentage of marker leakiness is comparable with that of cell lysis (Fig. 1), and (2) the composition of phospholipid species in the solubilized fraction is similar to that in the whole cell (data not shown). From the morphological studies, the solubilization by the two nonionic detergents may occur via membrane area expansion, and that by the two anionic detergents may be via vesiculation before cell lysis (Figs. 2F, 3F, 4E, 4F and 5F). However, the vesiculation in dodecylsulfate and in deoxycholate may be different, since the cell lysis in dodecylsulfate is parallel to the protein solubilization, while that in deoxycholate is parallel to the phospholipid solubilization (Figs. 1C and D). Also, in these two detergents, the lipid solubilities are different. The insertion of these detergents may exhibit different domain preference, since each component solubility is different in these detergents.

The rate of detergent uptake by platelet is determined by three factors: (1) the partition coefficient of the detergent between the membrane outer leaflet and aqueous phase; (2) the rate of flip-flop in membrane; and (3) the partition coefficient of the detergent between the inner leaflet and cytosol. Due to the asymmetric organization of membrane components in the membrane bilayer, these two partition coefficients may be different [38–43,24]. Also the flip-flop rates of various detergents may differ because of the molecular structure differences. Therefore, a time-course study of the effect of detergent on platelet morphology may reflect the transport of a detergent across the platelet membrane. In the lytic stage, platelets in various detergents require different incubation periods to attain complete solubilization. The order is octyl glucoside (less than 10 min) > deoxycholate (20 min) > Triton X-100 (30 min) ≥ dodecylsulfate (at least 30 min) (Table I). The same order is found in the morphological studies for the cells to change to their final shapes (Figs. 2–5). Hence, this order may reflect the different flip-flop phenomena of these detergents in the platelet membrane bilayer.

In the prelytic stage, the studies have demonstrated that platelets in these detergents show no increase in cytosolic free Ca^{2+} concentration (data not shown). Hence, the morphological change of platelets in these detergents may be a direct detergent effect (Figs. 2–5). The bilayer couple hypothesis been used to explain the

effect of membrane-active agent on platelet membrane morphology [20]. The spiculated platelet is due to an accumulation of the solute in the membrane outer leaflet, while the spherical platelet is due to the solute's concentrating in the inner leaflet. The study shows the time-dependent morphological change of platelets in various detergents. This may reflect the gradual change in the distribution of detergent between the two membrane leaflets and the flip-flop phenomenon. Specifically, the platelet morphology in octyl glucoside is as a sphere or a pear shape (Figs. 2D, E). The same kind of morphology is found in the chloropromazine-treated platelets [20]. Hence, the final octyl glucoside distribution between the two membrane leaflets may be more in the membrane inner leaflet than the outer one. In Triton X-100, the time-dependent morphological change may mean that Triton X-100 accumulates initially in the membrane outer leaflet, since the morphology at this stage is a spiculate disc. Due to a slow flip-flop rate of Triton X-100, it takes a longer incubation period (30 min) for the appearance of the final shape as a swollen disc with surface invaginations. This final morphology in Triton X-100 may mean that the concentration of Triton X-100 is greater in the inner leaflet than in the outer one. In the erythrocyte membrane, it has been reported that Triton X-100 accumulates more in the inner leaflet [44,45]. The platelets in dodecylsulfate and in deoxycholate appear to change from the initial spiculate disc to the final shape as an stretched-out flat form with pseudopods plus filopodia. Hence, these two anionic detergents may concentrate more in the outer leaflet than in the inner one. This phenomenon is also found in erythrocytes [44,45]. The morphological studies also show that these detergents can affect the organization of cytoskeletal molecules, since platelets in these detergents deformed to several different shapes (Figs. 2–5). It is conceivable that these detergents may diffuse into the cytosol to cause the reorganization of cytoskeletal molecules.

Platelets in these detergents all show inhibition of the response to collagen and ADP-fibrinogen, but no effect appears on the response to thrombin and phorbol ester (Table II). This may result from the perturbations of membrane by these detergents. However, it may also be true that these detergents inactivate collagen and ADP-fibrinogen, since the presumed membrane perturbations are not sufficient to decrease platelet aggregation in response to thrombin or phorbol ester, which could be explained if one assumes that the detergents do not denature thrombin or affect phorbol ester.

In summary, this study shows differences in the solubilization of platelets by various detergents. The detergents also cause different platelet morphological changes. These may be due to the asymmetric distribution of these detergents in the platelet membrane bilayer.

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