Biochimica et Biophysica Acta, 1028 (1990) 14–20
Elsevier

BBAMEM 74977

Surface exposure of phosphatidylserine is associated with the swelling and osmotically-induced fusion of human erythrocytes in the presence of Ca^{2+}

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(Received 14 February 1990)
(Revised manuscript received 29 May 1990)

Key words: Calcium ion; Cell fusion; Osmotic swelling; Phosphatidylserine; Phospholipid asymmetry; (Human erythrocyte)

An assay for procoagulant activity has been used to investigate the Ca^{2+}-dependent exposure of phosphatidylserine at the surface of human erythrocytes that were induced to swell and to fuse osmotically. Since the phosphatidylserine of human erythrocytes is located in the inner leaflet of the plasma membrane, it is inaccessible in intact cells which therefore had no procoagulant activity in an isotonic solution of sucrose. The procoagulant activity of erythrocytes incubated in increasingly hypotonic, sucrose solutions containing Mg^{2+} paralleled the percentage haemolysis, reflecting the accessibility of phosphatidylserine in an increasing number of lysed cells. However, cells in mildly hypotonic sucrose solutions containing Ca^{2+} had an abnormally high procoagulant activity indicating that phosphatidylserine was exposed in intact cells under these conditions. Erythrocytes that were subjected to continuous swelling at 37°C, which was induced by entry of the permeant molecule poly(ethylene glycol) 400 (PEG 400) developed procoagulant activity in the presence of Ca^{2+} prior to extensive lysis. Cells treated in this way also fused. With Mg^{2+}, PEG 400-treated erythrocytes lysed without fusing, and the development of procoagulant activity paralleled the rate of lysis. Erythrocytes incubated with ionophore A23187, subtilisin, and Ca^{2+} developed procoagulant activity (with < 20% lysis), and they fused on subsequent exposure to a hypotonic medium. The procoagulant activity reached its maximum before fusion could be induced in the hypotonic medium. It is concluded that the entry of Ca^{2+} facilitates a translocation of phosphatidylserine to the outer leaflet of the erythrocyte plasma membrane that plays an important role in fusion protocols that involve cell swelling. It is also suggested that transbilayer movements of phosphatidylserine could be an important control factor in the cell biology of membrane fusion phenomena.

Introduction

Hydrated bilayers of phospholipid normally repel each other strongly as a consequence of hydration repulsion arising from solvation of the polar head groups. When Ca^{2+} ions are added to phosphatidylserine, however, the surface water that gives rise to the repulsive forces is completely displaced by the bound Ca^{2+}, and the phospholipid bilayers achieve molecular contact [1,2]. Ca^{2+} ions are much more effective than other cations (e.g., Mg^{2+}) in inducing membrane fusion in model membrane systems based on phosphatidylserine [3] because the corresponding complex of phosphatidylserine with Mg^{2+} does not lose its layer of hydration [4]. Much experimental work has therefore been done on the Ca^{2+}-induced fusion of model membrane systems containing phosphatidylserine [4,5].

Some investigations on membrane fusion in biological systems have indicated that there may be an association between alterations in phospholipid asymmetry and membrane fusion [6–9] but, until very recently [10,11], relatively little attention has been paid to the need for phosphatidylserine to be on the outside surfaces of biological membranes if a phosphatidylserine-Ca^{2+} complex is required for them to come sufficiently close together for fusion to occur. The phospholipids in the outer leaflet of the human erythrocyte plasma mem-

Abbreviations: PEG, poly(ethylene glycol); S2238, *H*-D-phenylalanyl-L-pipecolyl-L-arginine-*p*-nitroanilide dihydrochloride; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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brane are primarily phosphatidylcholine and sphingomyelin, while the inner leaflet contains the acidic phospholipids [12]. In common with most cell types, erythrocytes do not exhibit spontaneous cell fusion reactions. On the above reasoning, this would be consistent with the topological distribution of phosphatidylserine in their plasma membranes, and it has been proposed that the presence of anionic phospholipids in the outer leaflet may be important in converting a fusion-incompetent to a fusion-competent membrane [13].

Exposure of phosphatidylserine on the outside of platelets [14,15] and sickled erythrocytes [16] yields procoagulant surfaces that facilitate the conversion of prothrombin into thrombin. The catalytic potential of such surfaces provides a semi-quantitative measure of the exposed phosphatidylserine [14,15]. Using this assay, we have found that the swelling and osmotically-induced fusion of human erythrocytes in the presence of Ca^{2+} is associated with the exposure of phosphatidylserine in the outer leaflet of the plasma membrane. These observations may be relevant to the molecular mechanisms of biomembrane fusion reactions that are of physiological and pathological importance.

Materials and Methods

Materials

Bovine blood coagulation factors (factor Xa, prothrombin, thrombin), poly(ethylene glycol) 400 (PEG 400) and Dextran (M_r 60000–90000) were from Sigma Chemical Co. Bovine factor V was from Diagnostic Reagents Ltd. The chromogenic substrate for thrombin, S2238 (*H*-D-phenylalanyl-L-pipecolyl-L-arginine-*p*-nitroanilide dihydrochloride) was from KabiVitrum (Stockholm, Sweden). Ionophore A23187 was from Eli Lilly and subtilisin BPN (Nagarse) was from Serva.

Methods

All assays were performed in duplicate and each experiment was undertaken at least three times. In each case, the results shown are from one representative experiment.

Assay of procoagulant activity and cell lysis

Phosphatidylserine was determined by an assay for prothrombinase activity [14]. Bovine factor V was activated at 35 $\mu g \cdot ml^{-1}$ in 10 mM Hepes buffer at pH 7.4, containing 2 mM $CaCl_2$, by incubation with 0.04 $\mu g \cdot ml^{-1}$ thrombin for 30 min at 37°C. Factor Xa, factor Va and $CaCl_2$ were added to cells or membranes in a solution of 110 mM NaCl and 40 mM Hepes buffer at pH 7.4. The mixture was incubated at 37°C for 2 min before the addition of prothrombin. Final concentrations in the assay were: $5 \cdot 10^6$–$2 \cdot 10^7$ ml^{-1} cells, 3 nM factor Xa, 6 nM factor Va, 4 mM $CaCl_2$, 2 μM prothrombin. At various times, thrombin formation was stopped by diluting a 20 μl aliquot of the incubation mixture into 0.5 ml of 50 mM Tris-HCl, 120 mM NaCl, 2 mM EDTA (pH 7.5) at room temperature. Chromogenic substrate S2238 was added to a final concentration of 150 μM, and the quantity of thrombin present was calculated from the rate of change of absorbance at 405 nm using a calibration curve. To determine the contribution made to the measured prothrombinase activity by unsealed red cell membranes, samples were centrifuged at $13000 \times g$ for 10 min, and cell lysis estimated from the absorbance at 414 nm of the haemoglobin in the supernatant. The absorbance values obtained were compared with those from a preparation of cells which had been totally lysed by freezing and thawing followed by sonication.

Cell swelling in hypotonic sucrose solutions

Human erythrocytes were obtained and washed as previously described [17] and then further washed in a 300 mM sucrose solution. Aliquots of packed cells were diluted 20-fold with a variety of hypotonic solutions of sucrose containing 10 mM $CaCl_2$ or 10 mM $MgCl_2$. The final osmolarities ranged from 41 to 195 mosM, and were checked with an osmometer. The erythrocytes were incubated in the hypotonic sucrose solutions for 45 min at 37° C. Hypotonic swelling was then reversed by diluting an aliquot of the cells 10-fold into ice cold 40 mM Hepes buffer at pH 7.4 containing 110 mM NaCl, and the cells were assayed for their prothrombinase activity and for the release of haemoglobin as described above.

Cell swelling in PEG 400

Aliquots of packed cells in 110 mM NaCl, 40 mM Hepes buffer (pH 7.4) were diluted 100-fold into a solution containing 20% (w/v) PEG 400 and 10 mM $MgCl_2$, with or without 10 mM $CaCl_2$. The cells were incubated at 37°C. At various times aliquots were removed and 1 M Hepes buffer (pH 7.4) and 4 M NaCl were added to give a final concentration of 40 mM Hepes and 110 mM NaCl. This halted the swelling caused by the entry of PEG 400. The incubation was then continued for a total of 120 min. Each sample was assayed for prothrombinase activity as described above, but with 20% (w/v) PEG 400 included in the assay mixture. The extent of cell lysis in each sample was also determined as above.

Cell fusion induced by PEG 400

The fusion of erythrocytes attached in monolayers to Alcian blue-coated plastic dishes was carried out essentially as previously described in experiments with PEG 300 [18].

Treatment of cells with ionophore A23187, Ca^{2+} and subtilisin

Erythrocytes in 40 mM Hepes buffer at pH 7.4, containing 110 mM NaCl, 80 mg · ml^{-1} Dextran (M_r 60000–90000), and 10 $CaCl_2$ were incubated in the presence or absence of ionophore A23187 (10 $\mu g \cdot ml^{-1}$) (dissolved in dimethyl sulphoxide at a concentration of 2 mg · ml^{-1}) with or without 50 $\mu g \cdot ml^{-1}$ subtilisin for 1 h at 37 °C. The extent of cell lysis and the procoagulant activity were then determined and compared with a fully lysed (frozen/thawed/sonicated) sample. Subtilisin had no effect on the assay at the concentrations used.

Fusion of cells treated with ionophore A23187 and Ca^{2+}

Monolayers of erythrocytes were attached to Alcian blue-coated plastic dishes as previously described [18]. The 40 mM Hepes buffer (pH 7.4), containing 110 mM NaCl, was replaced by 1 ml NaCl-Hepes buffer that contained 10 $\mu g \cdot ml^{-1}$ A23187, 10 mM $CaCl_2$, 50 $\mu g \cdot ml^{-1}$ subtilisin and 80 mg · ml^{-1} Dextran (M_r 60000–90000). The cells were incubated at 37 °C for 60–90 min. Cell fusion was then induced osmotically by diluting the medium with 2.0 ml of a solution of Dextran (80 mg · ml^{-1}).

Results

Procoagulant activity of erythrocytes in hypotonic sucrose solutions

In isotonic sucrose solutions, human erythrocytes had no procoagulant activity because phosphatidylserine in the inner leaflet of the plasma membrane is inaccessible in intact cells. Furthermore, when the cells were incubated for 45 min at 37 °C in sucrose solutions of decreasing osmolarity that contained 10 mM Mg^{2+}, their procoagulant activity paralleled the percentage lysis in the increasingly hypotonic solutions (Fig. 1). This pattern of response is presumably attributable to phosphatidylserine in the inner leaflet of the plasma membrane becoming accessible in an increasing number of cells as they lyse.

Contrasting observations were made with human erythrocytes which were incubated for 45 min at 37 °C in sucrose solutions of decreasing osmolarity that contained 10 mM Ca^{2+} (or 10 mM Ca^{2+} and 10 mM Mg^{2+}). In mildly hypotonic solutions containing Ca^{2+}, the cells exhibited an abnormally high procoagulant activity which was maximal in sucrose solutions that released only 15–30% of the haemoglobin (Fig. 2). This activity developed over about 30 min. The peak of abnormally high procoagulant activity which is illustrated in Fig. 2 was observed every time in eight separate experiments. These observations indicate that, when erythrocytes are incubated in mildly hypotonic sucrose solutions containing Ca^{2+}, phosphatidylserine is exposed in intact cells. In very dilute, sucrose solutions (40–60 mosM), the procoagulant activity of the lysed cells was relatively weak. It is suggested that all of the erythrocytes lysed very rapidly in the latter solutions, and that a proportion of the ghosts that were formed subsequently resealed.

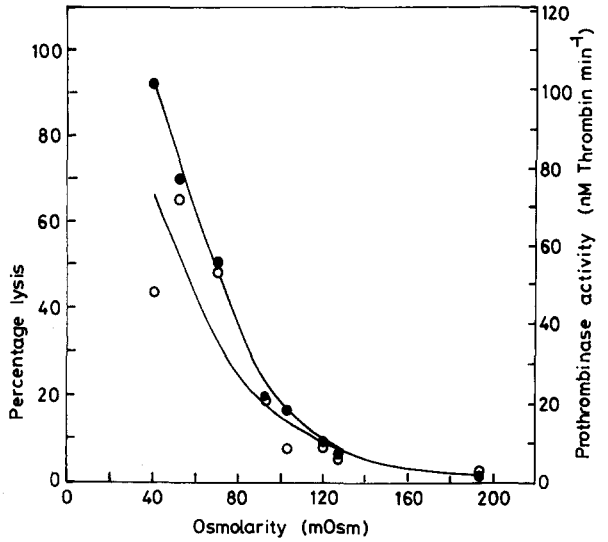

Fig. 1. Effect of cell swelling in hypotonic sucrose solutions on the surface exposure of phosphatidylserine in human erythrocytes. Cells were exposed to a series of sucrose solutions of decreasing osmolarity which contained 10 mM $MgCl_2$. The percentage lysis (●) and prothrombinase activity (○) were determined for each sample (see Materials and Methods). The prothrombinase activity for a totally lysed sample was 109.5 nM thrombin · min^{-1}.

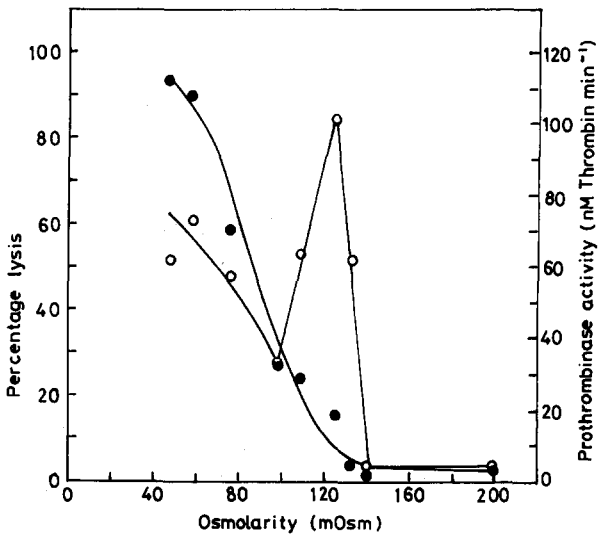

Fig. 2. Effect of cell swelling in hypotonic sucrose solutions containing Ca^{2+} on the surface exposure of phosphatidylserine in human erythrocytes. Cells were exposed to a series of sucrose solutions of decreasing osmolarity that contained 10 mM $CaCl_2$. The percentage lysis (●) and prothrombinase activity (○) were determined for each sample (see Materials and Methods). The prothrombinase activity for a totally lysed sample was 118.7 nM thrombin · min^{-1}.

It is known that erythrocytes that are subjected to osmotic swelling in a hypotonic salt solution swell and release some haemoglobin, and that the partially-haemolysed cells reseal as osmotic equilibrium is thus attained [19]. Such transient rupturing of the plasma membrane would be expected to allow extracellular ions, including Ca^{2+} to enter the cells. It is therefore considered that the abnormal procoagulant activity of intact cells in Ca^{2+}-containing, mildly hypotonic sucrose solutions (Fig. 2) results from the entry of extracellular Ca^{2+} ions, which induces a translocation of phosphatidylserine from the inner leaflet to the outer leaflet of the plasma membrane. This interpretation is consistent with the effect of diluting the contents of human erythrocytes in the presence of Ca^{2+} during the preparation of ghosts, which has been reported to cause randomization of the inner and outer leaflet phospholipids as judged by a changed affinity of the treated cells for the merocyanine dye MC540 [20]. It is unlikely that the abnormal procoagulant activity seen in Fig. 2 resulted from entry into the treated erythrocytes of reagents for the assay of prothrombinase activity since this would require factor Va, which has a relative molecular weight of 330 000, to have entered the swelling cells before haemoglobin (M_r 65 000) was released.

Procoagulant activity and fusion of PEG 400-treated cells

It has previously been shown that, in the presence of Ca^{2+}, human erythrocytes fuse before they lyse when the cells swell in solutions of permeable molecules such as malonamide and PEG 300, in which a stable osmotic equilibrium is not obtainable. When Ca^{2+} is absent or is replaced by Mg^{2+}, the swelling cells lyse without fusing [18].

Experiments were done to investigate the effect of Ca^{2+} on the exposure of phosphatidylserine in human erythrocytes that were incubated for 2 h at 37°C in a 20% (w/v) solution of PEG 400 containing 10 mM Mg^{2+}. Mg^{2+} was included in the PEG solution since it greatly reduced aggregation of the cells. Mg^{2+} also decreased lysis at early time points, probably by facilitating resealing. (It was convenient to use PEG 400 in the present work since swelling and cell fusion occurred more slowly than with the PEG 300 used previously [18]; malonamide [18] could not be used in the present work because it inhibited the prothrombinase assay.) Fig. 3 (panel A) shows that the development of coagulant activity preceded cell lysis in the presence of Ca^{2+}. By contrast, in the absence of Ca^{2+}, the development of procoagulant activity paralleled the rate of lysis (Fig. 3, panel B).

Monolayers of erythrocytes that were incubated with 20% PEG 400 in the presence of 10 mM Ca^{2+}, with or without Mg^{2+}, exhibited extensive fusion after 45–60 min (Fig. 4). In the absence of Ca^{2+}, the swelling cells merely lysed.

In previous work [18], it was shown that the swelling and lysis of human erythrocytes, which occurs on incubation with the permeable molecule PEG 300, is inhibited by the presence of larger molecules such as raffinose, sucrose, and serum albumin. Inclusion of serum albumin in the incubation medium also inhibited the cell fusion which occurred when Ca^{2+} was present. We have now observed that the addition of bovine serum albumin (60 mg · ml^{-1}) to human erythrocytes, after 25 min of incubation at 37°C with PEG 400 and Ca^{2+}, inhibits further swelling and fusion. This additionally demonstrates that serum albumin (M_r 67 000)

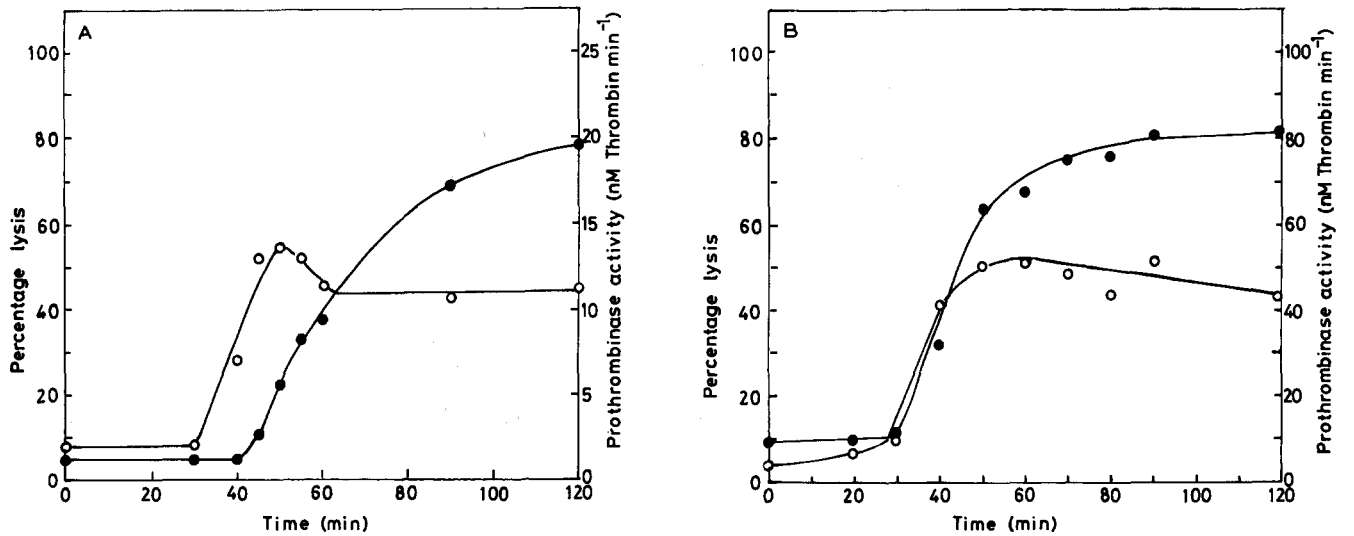

Fig. 3. Effect of swelling human erythrocytes in a solution of PEG 400 on the surface exposure of phosphatidylserine. Cells were incubated in 20% (w/v) PEG 400 containing 10 mM $CaCl_2$ and 10 mM $MgCl_2$ (panel A), or 10 mM $MgCl_2$ (panel B). At various times cell swelling was halted, and the percentage lysis (●) and prothrombinase activity (○) were determined (see Materials and Methods). The prothrombinase activities for totally lysed samples were 23 nM thrombin · min^{-1} for panel A, and 92.2 nM thrombin · min^{-1} for panel B.

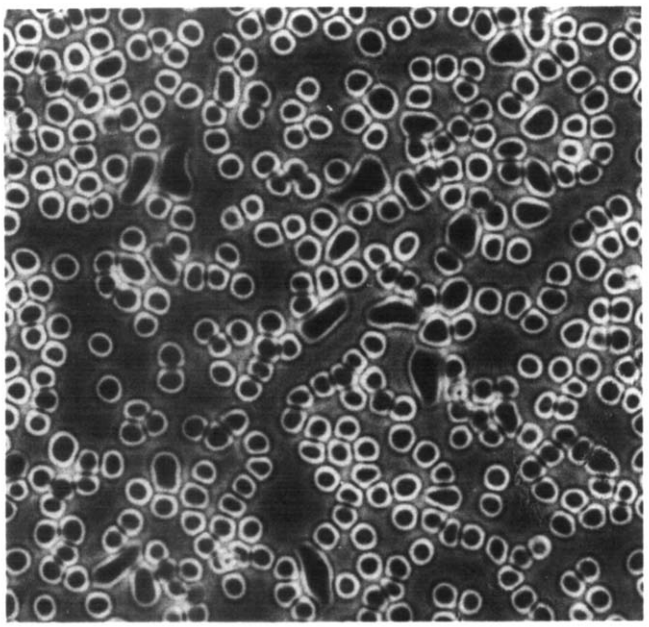

Fig. 4. Phase-contrast, light micrograph of human erythrocytes showing cell fusion induced by treatment with PEG 400. A monolayer of cells was incubated at 37°C for 60 min with 20% (w/v) PEG 400 containing 10 mM $MgCl_2$ and 10 mM $CaCl_2$ (see Materials and Methods). Magnification: ×200.

does not enter the treated cells, and again indicates that the much larger factor Va which is used in the procoagulant assay is also unlikely to enter the treated cells.

Procoagulant activity and fusion of ionophore A23187-treated cells

Early studies showed that erythrocytes fuse on incubation with ionophore A23187 and Ca^{2+} [21,22]. This treatment thus provides an alternative way of investigating the relationships between Ca^{2+} entry, the exposure of phosphatidylserine, and cell fusion. Unlike hen erythrocytes [21], human erythrocytes do not fuse with ionophore and Ca^{2+} unless subtilisin is also present [22]. Human erythrocytes were therefore incubated at 37°C for 1 h with A23187 (10 $\mu g \cdot ml^{-1}$), Ca^{2+} (10 mM) and subtilisin (50 $\mu g \cdot ml^{-1}$). From Table I it may be seen that the cells developed procoagulant activity in the presence of Ca^{2+} and A23187, but not when the ionophore was absent. The procoagulant activity was increased by subtilisin, and it reached a maximum in about 30 min in the presence of the enzyme. It is not clear why procoagulant activity was increased by subtilisin, but it may be relevant that subtilisin can apparently inhibit the inward transport of phosphatidyl-

TABLE I

Effect of ionophore A23187, Ca^{2+}, and subtilisin on the surface exposure of phosphatidylserine in human erythrocytes

Cells in 40 mM Hepes buffer (pH 7.4) containing 110 mM NaCl, 80 $mg \cdot ml^{-1}$ Dextran (M_r 60000–90000), and 10 mM $CaCl_2$ were incubated as described in Materials and Methods. For each sample, the release of haemoglobin and the prothrombinase activity were compared as percentages with the values obtained from a fully lysed sample that had a prothrombinase activity of 38.2 nM thrombin · min^{-1}.

Treatment	% lysis	% prothrombinase activity
Control incubation	8.5	3.4
50 $\mu g \cdot ml^{-1}$ subtilisin	10.0	6.7
10 $\mu g \cdot ml^{-1}$ A23187	10.4	36.6
50 $\mu g \cdot ml^{-1}$ subtilisin and 10 $\mu g \cdot ml^{-1}$ A23187	16.0	66.2
Lysis by freezing, thawing and sonication	100	100

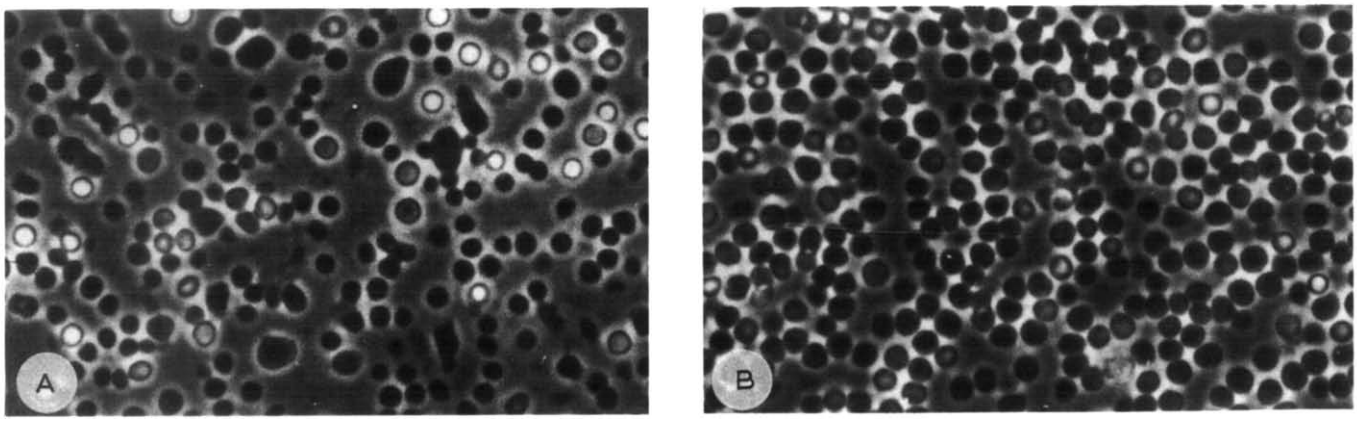

Fig. 5. Fusion of human erythrocytes induced to fuse by treatment with ionophore A23187, Ca^{2+}, and subtilisin, followed by an osmotic shock. For panel A, a monolayer of cells was incubated at 37°C for 90 min with 1.0 ml of 40 mM Hepes buffer (pH 7.4) containing 110 mM NaCl, 10 mM $CaCl_2$, 80 $mg \cdot ml^{-1}$ Dextran (M_r 60000–90000), 10 $\mu g \cdot ml^{-1}$ A23187, and 50 $\mu g \cdot ml^{-1}$ subtilisin. Cell fusion was then induced osmotically by diluting the medium with 2.0 ml of a solution of Dextran (80 $mg \cdot ml^{-1}$). The micrograph was taken 30 min later. For panel B, the cells were treated as for panel A but without ionophore A23187 and subtilisin. (Erythrocytes treated with subtilisin in the absence of A23187 aggregated extensively but did not fuse.) Magnification: ×200.

serine that has been exposed at the surface of stimulated platelets [23].

Cells that were incubated for 60–90 min with Ca^{2+}, ionophore A23187, and subtilisin, and were then subjected to an osmotic shock, began to fuse after 10 min. Extensive fusion was seen after 30 min although a high proportion of the cells also lysed (Fig. 5, panel A). In the absence of the ionophore, no fusion occurred within 30–40 min of the osmotic shock (Fig. 5, panel B).

In experiments with ionophore A23187, it was not possible to check whether or not the replacement of Ca^{2+} by Mg^{2+} resulted in the formation of a procoagulant surface since it is necessary to add Ca^{2+} for the prothrombinase assay. The added Ca^{2+} then immediately enters the treated cells. However, no fusion occurred following incubation of erythrocytes with A23187 and Mg^{2+}. The prothrombinase activity observed under such conditions was intermediate between the activity observed with Ca^{2+} plus ionophore, and the activity with Ca^{2+} but no ionophore.

Discussion

From early investigations on chemically-induced cell fusion, it was concluded that cell swelling plays an essential role in the action of fusogenic lipids on erythrocytes [24]. Fusion induced by these agents was also found to be Ca^{2+}-dependent [25], and associated with the entry of extracellular Ca^{2+} into the treated cells [26]. Other work demonstrated that erythrocytes fuse following incubation with Ca^{2+} in the presence of the divalent cation ionophore A23187 [21,22].

Erythrocytes incubated with Ca^{2+} and A23187 have now been found to develop procoagulant activity. The presence of Ca^{2+} ions in mildly hypotonic sucrose solutions also enabled partially-haemolysed, but still intact erythrocytes to develop procoagulant activity. In addition, erythrocytes that were exposed to the permeable molecule, PEG 400, developed procoagulant activity in the presence of Ca^{2+}, and monolayers of erythrocytes treated with PEG 400 and Ca^{2+} were induced to fuse. These several observations indicate that the entry of extracellular Ca^{2+}, which results either from treatment with ionophore A23187 or from rupture and resealing of the plasma membrane in swelling cells, leads both to exposure of phosphatidylserine in the outer leaflet of the erythrocyte plasma membrane and to cell fusion.

Two factors are involved in the regulation of phospholipid asymmetry in the plasma membrane of erythrocytes. Some experiments indicate that spectrin functions to stabilize anionic phospholipids in the inner leaflet of the bilayer, and that when the spectrin network is disrupted a loss of asymmetry ensues [27,28]. Other work indicates that the asymmetry is maintained primarily by an ATP-dependent aminophospholipid translocase [29]. Studies on sickled erythrocytes have, however, indicated that the asymmetric distribution of phosphatidylserine depends on both of these factors [30].

In platelets, the development of procoagulant activity parallels a degradation of their cytoskeletal proteins (filamin and talin) by calpain which occurs when the cells are activated by Ca^{2+} and ionophore A23187 [15,16]. It has therefore been proposed that an interaction of phosphatidylserine with the cytoskeleton of platelets minimizes its outward movement (thus reducing ATP consumption by the translocase) and that, when an increased exposure of phosphatidylserine is required for haemostasis, both translocase activity and the cytoskeletal interaction are rapidly and temporarily disturbed [23]. Previous work on the chemically-induced fusion of erythrocytes has shown that the activity of an endogenous, Ca^{2+}-dependent thiol-proteinase (calpain), and proteolytic degradation of the cytoskeletal network, are involved in the fusion process [17,32–34]. Consequently, it seems probable that a Ca^{2+}-dependent, proteolytic degradation breakdown of structural components of the plasma membrane mediates the exposure of phosphatidylserine at the cell surface that has been found to be associated with cell fusion in the present paper.

There have been previous indications that changes in the phospholipid asymmetry of biomembranes may be a feature of membrane fusion reactions. For example, the outer leaflet of the myoblast plasma membrane is unusually rich in both phosphatidylserine and phosphatidylethanolamine, and it has been suggested that this may be germane to the fusion of myoblasts [6]. However, it seems that the eccentric distribution of aminophospholipids may not actually determine fusion competence in myoblasts [7]. Some 40% of the phosphatidylserine and 60% of the phosphatidylethanolamine are on the cytoplasmic surface of synaptic vesicles [8], and it has recently been reported that an ATP-dependent aminophospholipid translocase selectively transports phosphatidylserine from the luminal to the cytoplasmic monolayer in chromaffin granules [11]. These latter findings may be relevant to membrane fusion occurring in exocytosis.

With erythrocytes, the almost exclusive localisation of phosphatidylethanolamine in the inner leaflet is completely lost in ghosts prepared by colloid-osmotic lysis after electric breakdown and resealing. Phosphatidylserine was much less affected but it was suggested that an enhanced mobility of phosphatidylethanolamine may facilitate the merging of two closely apposed bilayers [9]. During the present investigation, it has been reported that human erythrocytes submitted to a 'preswell lysis and resealing procedure' in the presence of Ca^{2+} [20] exhibit an increased susceptibility to fusion by high molecular weight poly(ethylene glycol) (PEG 6000) [10]. Since this treatment apparently causes a loss

of phospholipid asymmetry, it has been concluded that lipid-symmetric erythrocytes fuse relatively readily on treatment with PEG 6000 [10]. However, our observations would seem to be the first direct demonstration that cell fusion reactions, albeit artificially-induced, are associated with a change in the location of phosphatidylserine in the plasma membranes of the fusing cells.

The surface exposure of phosphatidylserine in fusing erythrocytes that is reported here provides some experimental justification for the use of symmetrical phospholipid vesicles, which contain phosphatidylserine, as models for the study of biomembrane fusion. Our observations are also consistent with the idea that phosphatidylserine needs to be in the outer leaflet in biomembrane fusion reactions, so that a phosphatidylserine-Ca^{2+} complex can be formed which allows membranes to come sufficiently close together for fusion to occur. Transbilayer movements of phosphatidylserine may indeed be important in vivo as a control factor in membrane fusion phenomena. In addition, it is conceivable that in some cases osmotically-driven swelling not only gives rise to the translocation of phosphatidylserine which allows membranes to make contact, but also subsequently provides the driving force for the fusion reaction itself [35,36].

Acknowledgements

We thank the Medical Research Council for the award of a Project Grant that supported this work.

References

1 Parsegian, V.A., Rand, R.P. and Gingell, D. (1984) in Cell Fusion (Evered, D. and Whelan, J., eds.), pp. 9–27, Pitman, London.
2 Coorssen, J. and Rand, R.P. (1988) Stud. biophys. 127, 53–60.
3 Levintis, R., Gagne, J., Fuller, N., Rand, R.P. and Silvius, J.R. (1986) Biochemistry 25, 6978–6987.
4 Düzgünes, N. (1985) in Subcellular Biochemistry (Roodyn, D.B., ed.), Vol. 11, pp. 195–286, Plenum Press, New York.
5 Papahadajopoulos, D. (1978) in Membrane Fusion (Poste, G. and Nicolson, G.L., eds.), pp. 765–790, North-Holland, Amsterdam.
6 Sessions, A. and Horwitz, A.F. (1981) FEBS Lett. 134, 75–78.
7 Horwitz, A., Neff, N., Sessions, A., and Decker, C. (1982) in Muscle Development: Molecular and Cellular Control (Pearson, M.L. and Epstein, H.F., eds.), pp. 291–300, Cold Spring Harbor Laboratory, Cold Spring Harbor.
8 Deutsch, J.W. and Kelly, R.B. (1981) Biochemistry 20, 378–385.
9 Dressler, V., Schwister, K., Haest, C.W.M. and Deuticke, B. (1983) Biochim. Biophys. Acta 732, 304–307.
10 Tullius, E.K., Williamson, P. and Schlegel, R.A. (1989) Biosci. Rep. 9, 623–633.
11 Zachowski, A., Henry, J.-P. and Devaux, P.F. (1989) Nature 340, 75–76.
12 Zwaal, R.F.A. (1978) Biochim. Biophys. Acta 515, 163–205.
13 Schlegel, R.A. and Williamson, P. (1988) in Molecular Mechanisms of Membrane Fusion (Ohki, S., Doyle, D., Flanagan, T.D., Hui, S.W. and Mayhew, E., eds.), pp. 289–301, Plenum Press, New York.
14 Verhallen, P.F.J., Bevers, E.M., Comfurius, P. and Zwaal, R.F.A. (1987) Biochim. Biophys. Acta 903, 206–217.
15 Verhallen, P.F.J., Bevers, E.M., Comfurius, P. and Zwaal, R.F.A. (1988) Biochim. Biophys. Acta 942, 150–158.
16 Middelkoop, E., Lubin, B.H., Bevers, E.M., Op den Kamp, J.A.F., Comfurius, P., Chiu, D.T.-Y., Zwaal, R.F.A., Van Deenen, L.L.M. and Roelofsen, B. (1988) Biochim. Biophys. Acta 937, 281–288.
17 Quirk, S.J., Ahkong, Q.F., Botham, G.M., Vos, J. and Lucy, J.A. (1978) Biochem. J. 176, 159–167.
18 Ahkong, Q.F. and Lucy, J.A. (1988) J. Cell Sci. 91, 597–601.
19 Lowenstein, L.M. (1960) Exp. Cell Res. 20, 56–65.
20 Williamson, P., Algarin, L., Bateman, J., Choe, H.-R. and Schlegel, R.A. (1985) J. Cell. Physiol. 123, 209–214.
21 Ahkong, Q.F., Tampion, W. and Lucy, J.A. (1975) Nature 256, 208–209.
22 Ahkong, Q.F., Blow, A.M.J., Botham, G.M., Launder, J.M., Quirk, S.J. and Lucy, J.A. (1978) FEBS Lett. 95, 147–152.
23 Bevers, E.M., Tilly, R.H.J., Senden, J.M.G., Comfurius, P. and Zwaal, R.F.A. (1989) Biochemistry 28, 2382–2387.
24 Ahkong, Q.F., Cramp, F.C., Fisher, D., Howell, J.I., Tampion, W., Verrinder, M. and Lucy, J.A. (1973) Nature New Biol. 242, 215–217.
25 Ahkong, Q.F., Fisher, D., Tampion, W. and Lucy, J.A. (1973) Biochem. J. 136, 147–155.
26 Blow, A.M.J., Botham, G.M. and Lucy, J.A. (1979) Biochem. J. 182, 555–563.
27 Haest, C.W.M., Plasa, G., Kamp, D. and Deuticke, B. (1978) Biochim. Biophys. Acta 509, 21–32.
28 Williamson, P., Bateman, J., Kozarsky, K., Mattocks, K., Hermanowicz, N., Choe, H.-R. and Schlegel, R.A. (1982) Cell 30, 725–733.
29 Calvez, J.-Y., Zachowski, A., Herrmann, A., Morrot, G. and Devaux, P.F. (1988) Biochemistry 27, 5666–5670.
30 Middelkoop, E., Lubin, B.H., Bevers, E.M., Op den Kamp, J.A.F., Comfurius, P., Chiu, D.T.-Y., Zwaal, R.F.A., Van Deenen, L.L.M. and Roelofsen, B. (1988) Biochim. Biophys. Acta 937, 281–288.
31 Ahkong, Q.F., Botham, G.M., Woodward, A.W. and Lucy, J.A. (1980) Biochem. J. 192, 829–836.
32 Lang, R.D.A., Wickenden, C., Wynne, J. and Lucy, J.A. (1984) Biochem. J. 218, 295–305.
33 Glaser, T. and Kosower, N.S. (1986) FEBS Lett. 206, 115–120.
34 Lucy, J.A. and Ahkong, Q.F. (1986) FEBS Lett. 199, 1–11.
35 Lucy, J.A. and Ahkong, Q.F. (1988) in Molecular Mechanisms of Membrane Fusion (Ohki, S., Doyle, D., Flanagan, T.D., Hui, S.W. and Mayhew, E., eds.), pp. 163–179, Plenum Press, New York.