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Lipid composition of subcellular particles from sheep platelets. Location of phosphatidylethanolamine and phosphatidylserine in plasma membranes and platelet liposomes

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The lipid composition of whole sheep platelets and their subcellular fractions was determined. The basic lipids show similar distributions in granules, microsomes, plasma membranes and whole platelets. Phospholipid (about 70% of total lipids) and cholesterol (25% of total lipids) are the principal lipid components. Free cholesterol represents about 98% of the total, whereas cholesteryl ester is a minor component. The phospholipid composition found in intact platelets and their subcellular particles is about: 35% phosphatidylethanolamine (PE), 30% phosphatidylcholine (PC), 20% sphingomyelin and 15% phosphatidylserine (PS). We also investigated aminophospholipid topology in intact platelet plasma membranes and platelet liposomes by using the nonpenetrating chemical probe trinitrobenzenesulfonic acid (TNBS), because they are the major components of total lipids. In intact platelets, PS is not accessible to TNBS during the initial 15 min of incubation, whereas 18% PE is labelled after 15 min. In contrast, in phospholipid extracted from platelets 80% PE and 67% PS react with TNBS within 5 min, while 27 and 25% PE and 15 and 19% PS from liposomes and isolated plasma membranes, respectively, were modified after 15 min of incubation. In view of this chemical modification, it is concluded that 22% of PE and less than 1% of PS are located on the external surface of intact platelet plasma membranes. The asymmetric orientation of aminophospholipids is similar between liposomes and isolated plasma membrane. PS (23 and 28%) and PE (34 and 31%) are scarcely represented outside the bilayer. The data found are consistent with the nonrandom phospholipid distribution of blood cell surface membranes.

Introduction

Platelet lipids are thought to be critical components of platelet activity in normal platelet aggregation and in thrombosis [1].

The lipid composition of platelets from several species has been characterized [2–6], and phospholipids represent the major constituent of the platelet plasma membrane [2–4]. In addition to the structural function of the platelet lipids, they have been shown to participate in various platelet responses, such as providing a phospholipid/water interface to interacting coagulation factors [1], and their possible involvement in the platelet release reaction as a storage site for arachidonic acid, a precursor in prostaglandin biosynthesis [7,8]. The

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SDS, sodium dodecyl sulfate; TLC, thin-layer chromatography; TNBS, 2,4,6-trinitrobenzenesulfonate.

localization of lipids in biological membranes has primarily been carried out via chemical or enzymatic modification, via exchange techniques, and in some cases by immunochemical procedures. Physicochemical approaches have been used mainly in studies on model membrane systems. A specific reagent for amino groups, trinitrobenzenesulfonate, has been widely used to localize aminophospholipids in biological membranes of different origin such as erythrocytes [10–12], platelets [13], bacteria [14,15], viruses [16], fibroblasts [17], sarcoplasmic reticulum [18], liposomes [19] and membranes from various subcellular organelles [20,21]. Furthermore, independent evidence for the phospholipid asymmetric distribution in blood cells was obtained with phospholipases [22–24]. This research has led to the conclusion that the bilayer is asymmetric. Phospholipid orientation in erythrocyte and platelet plasma membranes appears to be rather similar for both types of membranes. The negatively charged phospholipids are almost exclusively located on the inner leaflet. This provides an explanation for the procoagulant activity of the inner surface of both membranes because PS is the most active coagulant phospholipid; it represents a safety mechanism to protect the circulation from possible hypercoagulation and thrombosis [9].

The topology of different mixed phospholipids has been studied mainly in large multilamellar and unilamellar vesicles. However, the phospholipid arrangement in artificial membranes obtained from lipid platelets is still unknown. The aim of the experiments described in this paper was to shed light on the aminophospholipid distribution in sheep platelet plasma membranes and in artificial vesicles prepared with lipids extracted from these platelets; such studies were based on the use of the trinitrobenzenesulfonic acid. The lipid composition of intact platelets and their subcellular fractions were also established.

Materials and Methods

Materials. The following materials were used in the experimental procedure: phenylmethylsulfonyl fluoride (PMSF), 4-amino-3-naphtol-1-sulfonic acid, octyl-β-D-glucopyranoside, Tris (hydroxymethyl) aminomethane and bovine serum albumin were purchased from Sigma (St. Louis, MO, U.S.A.). D-Sucrose was from Aldrich-Europe (Beerse, Belgium). Phospray and lipid standards were obtained from Supelco, S.A. (1196 Gland, Switzerland) and the 2,4,6-trinitrobenzenesulfonate from Serva Feinbiochemica (Heidelberg, F.R.G.). The thin-layer chromatography plates (Silica gel G, type 60) were from Merck (E. Merck, Darmstadt, F.R.G.). All organic solvents were of high quality and glass-distilled. The remaining products were supplied by Probus (Spain).

Preparation of platelets. Platelets were isolated from freshly collected blood of adult sheep (*Ovis aries* L. var domestica) by the procedure previously described [25]. The blood was obtained from a local slaughterhouse and colected into 500-ml polythene vessels containing 75 ml 1.5% disodium EDTA in 0.15 M NaCl as anticoagulant. These platelets were used for the study of their lipid composition. Location of aminophospholipids in platelet plasma membranes was performed with platelets obtained following the procedure of Barber and Jamieson [26], slightly modified. The platelet preparation was precipitated at $3000 \times g$ for 30 min, then gently suspended in solution 3 (40 mM KCl/120 mM $NaHCO_3$/5.5 mM glucose) (pH 8.5–8.6) and washed once in the same solution.

Isolation of subcellular fractions. Three main subcellular fractions were isolated from whole platelets by using a discontinuous sucrose gradient under similar conditions to those indicated by Llanillo and Cabezas [25]. The characterization of these fractions was described in a previous paper [25]. The Bp fraction was enriched in plasma membranes. The second fraction (Cp) consisted of some granules (lysosomes and mitochondria) and membranes from the endoplasmic reticulum. Most of the intracellular granules were included in the third fraction (Dp).

The protein content of different fractions was determiend by the method of Lowry et al. [27].

Preparation of lipid vesicles (liposomes). Total lipids extracted from whole platelets were used to produce large lipid vesicles by a detergent-dialysis method [28]. Enough lipids to provide 1–2 ml of a suspension with a concentration of 1 mM in lipid phosphorus were solubilized with octyl-β-D-glucopyranoside (15 mg of detergent/mg lipid phos-

phorus) in a vesicle buffer (10 mM Tris/262 mM NaCl/5 mM KCl/1 mM EDTA/0.02% NaN_3) (pH 7.4). The detergent/lipid mixture was extensively dialyzed at 4°C against a cooling vesicle buffer (5 × 1 liter changes within 60 h), and large lipid vesicles were formed as the detergent was removed. In order to eliminate the lipid components not incorporated into the lipid vesicles after dialysis, the sample was loaded on a Sephacryl S-1000 column (0.9 × 50 cm), previously preequilibrated with sonicated platelet lipid (5 mg/ml vesicle buffer) to prevent lipid adsorption on the gel matrix. The column was eluted with vesicle buffer, monitored by recording the scattering due to the liposomes at 335 nm, and maintained at room temperature throughout the run. The preparations were stored under nitrogen, in order to prevent the loss of vesicle integrity.

Lipid extraction, fractionation and identification. Total lipids from whole platelets and subcellular platelet particles were extracted according to the method of the Bligh and Dyer [29]. Lipid extracts were washed with a 0.73% NaCl solution and evaporated to complete dryness. The final lipid residues were determined gravimetrically, dissolved down to approx. 25 mg of lipid/ml in chloroform/methanol (2 : 1, v/v), and stored under nitrogen at −20°C until analysis.

Total cholesterol of lipid extracts was evaluated by the method of Zlatkis et al., as reported by Kates [30]. Lipid phosphorus was determined after hydrolysis with a mixture of $HClO_4$ and H_2SO_4 by the Bartlett procedure [31].

Neutral lipid classes were fractionated by thin-layer chromatography (TLC) on activated 0.25-mm plates of Silica gel 60G (Merck) using hexane/ethyl ether/acetic acid/methanol (60 : 40 : 1 : 1, v/v) as the developing solvent. The components were visualized after treatment with iodine and identified by comparison with standards. The stained areas corresponding to free cholesterol and cholesteryl esters, as well as control blanks, were scraped off, after which the cholesterol concentration in each sample was determined by the method reported above [30].

Aliquots containing 6–7 μg of lipid phosphorus were applied on activated 10 × 20 cm plates (0.25 mm) of Silica gel 60G, and developed twice using chloroform/methanol/conc. ammonia (65 : 24 : 4, v/v) first, and then chloroform/acetone/methanol/acetic acid/water (30 : 40 : 10 : 10 : 5, v/v). The material labelled by TNBS was also fractionated by two-dimensional TLC on activated 20 × 20 cm Silica gel 60G plates, but using chloroform/methanol/water (65 : 25 : 4, v/v) in the first dimension. The lipids which reacted with TNBS were identified by their yellow color, and the free lipids were located using iodine vapors, or by spraying with Phospray (for detection of phosphorus-containing lipids). Phospholipid species were identified by comparing their migration with phospholipid standards. Each spot was scraped, digested and their lipid phosphorus containing analyzed [31], in order to assess the percentage of different phospholipid species.

Labelling conditions with TNBS. The labelling procedure was based on the methods of Gordesky and Marinetti [10], Sandra and Pagano [17] and Vale [18]. The extracted lipids from platelets (1 μg of lipid phosphorus/ml incubation medium) were evaporated to dryness under nitrogen, and solubilized in an incubation medium (50 mM KCl/100 mM $NaHCO_3$) (pH 8.5–8.6) by octyl-β-D-glucopyranoside (15 mg of detergent/mg lipid phosphorus). Before the modification assay, whole platelets, isolated platelet plasma membranes and lipid vesicles were resuspended in the incubation medium after which the TNBS (buffered with 5% $NaHCO_3$) was added to a final TNBS/lipid phosphorus molar ratio of 35. The different mixtures were incubated at room temperature for 5, 15, 30, 60, 90 or 120 min, and the reaction was stopped by the addition of 0.5 ml of solution (1 M HCl/0.5% SDS) per 2.5 ml of each mixture. Labelled lipids from solubilized lipids, and isolated platelet membranes or liposomes were extracted and well-separated as described above. The labelled platelets were collected by centrifugation at 4300 × *g* for 10 min, and washed twice with buffered solution 4 (10 mM potassium phosphate/5.5 mM glucose/0.140 M NaCl) (pH 7.0). Their particulate fractions were obtained by centrifugation of platelet homogenate at 105 000 × *g* for 1 h at 4°C, resuspended in buffered solution 4 (pH 7.0), and subjected to lipid extraction.

Statistics. The Scheffe test following variance analysis (when the differences were significant) was employed to compare the various grouped results.

Results and Discussion

Lipid composition of whole platelets and their subcellular fractions

The lipid composition of sheep platelets has not been previously reported, and preliminary experiments were aimed at determining the individual classes of lipids in this material.

The crude lipids of whole platelets and their platelet subcellular fractions were obtained from the Folch organic phase; the data are shown in Table I. These results demonstrate that a similar lipid distribution exists in whole platelets, plasma membranes, Cp and Dp fractions. The main lipid classes are phospholipid (about 70% of total lipids) and total cholesterol (about 25%). The lipid-to-protein ratio was higher in plasma membranes than in other fractions, and is in close agreement with previously published results in other materials [2,26]. Highly specialized cell membranes which are metabolically active have a high protein content as compared with lipids [32]. The cholesterol (0.45 ± 0.04 μmol/mg protein) and phospholipid (0.63 ± 0.05 μmol/mg protein) concentration of the plasma membrane fraction was about twice as high as that of the Cp and Dp fractions; these differences are statistically significant ($P < 0.01$). Similar findings were obtained in human [3] and pig platelets [33], respectively. We have not found statistically significant differences in the cholesterol/phospholipid molar ratio between the platelet plasma membranes (0.77) and other subcellular fractions. However, the cholesterol/phospholipid molar ratio in sheep platelet plasma membranes was higher than that found in human platelet membranes [2,26]. These data agree with the results obtained in pig platelets [33,4] and erythrocyte ghosts [32].

The phospholipid composition is summarized in Table II. PE, PC, PS and sphingomyelin are always the major phospholipid components.

Percentages were calculated by referring to the total amount of the lipid phosphorus from the four major components at 100%. Cardiolipin, phosphatidic acid, PI, lysoPC and lysoPE were considered as trace amounts (2–0.5%). The most abundant phospholipids in all fractions from sheep platelets are aminophospholipids (about 50%), which are present in higher amounts than the PC content, the predominant phosphatide in cell membranes from human and pig platelets [2,4,23, 24]. The phospholipids found in sheep platelets and their subcellular fractions are about: 35% of PE, 30% of PC, 20% of sphingomyelin and 15% of PS. There are no statistically significant differences ($P > 0.05$) between aminophospholipid percentages from whole platelets and their different fractions. However, the PC percentages from both plasma membranes and Cp fractions as well as sphingomyelin percentages from platelets compared with all subcellular fractions show statistically significant differences ($P < 0.05$).

The major component of neutral lipids is free cholesterol; it represents about 98.5% of total

TABLE I

TOTAL LIPID CONTENTS IN SHEEP PLATELETS

Total lipids were determined gravimetrically. Proteins, lipid phosphorus and cholesterol were assayed by colorimetric procedures (see Materials and Methods). The phospholipids were calculated by assuming 25 μg phospholipid/μg lipid phosphorus, and 780 as molecular weight. The values presented are means ± S.D., obtained in four assays.

Fraction	Component				
	lipid/protein w/w	phospholipid/ protein (μmol/mg)	cholesterol/ protein (μmol/mg)	phospholipid/ total lipid (%)	cholesterol/ total lipid (%)
Whole platelets	0.27 ± 0.03	0.26 ± 0.01	0.15 ± 0.04	70.3 ± 3.0	24.5 ± 2.6
Plasma membranes (Bp)	0.53 ± 0.06	0.63 ± 0.05	0.45 ± 0.04	67.6 ± 2.6	26.0 ± 2.6
Intracellular membrane + granules (Cp)	0.37 ± 0.03	0.32 ± 0.03	0.26 ± 0.03	65.2 ± 3.6	25.7 ± 3.0
Granules (Dp)	0.35 ± 0.08	0.27 ± 0.03	0.22 ± 0.05	69.9 ± 4.0	21.3 ± 2.0

cholesterol from whole platelets and their subcellular fractions, and 25% of total lipids (see Table III). Our findings are in accordance with previous results in human platelets [2] and also with the suggestion of Bloch, that free cholesterol in cells is mainly associated with intracellular and plasma membranes [34]. The cholesteryl esters represent approx. 0.4% of total lipids (2% of total cholesterol, Table III). Triacylglycerols, free fatty acids, diacylglycerols and cerebrosides are included in the group of remaining lipids, which could not be identified owing to their low concentrations. However, neither whole platelets nor subcellular particles are characterized by a specific lipid.

The aminophospholipids represent the major lipid component in all particulate fractions (46 and 52% of the membrane phospholipids and phospholipid extracted from whole platelets, respectively). They could be linked, at least indirectly, to many aspects of platelet activity. We have investigated the topology of aminophospholipids in sheep platelet plasma membranes and lipid vesicles, since an asymmetry in lipid organization would explain the platelet membrane properties (changes of shape, adhesion, secretion, etc.), with no apparent change in lipid composition. The availability of a convenient model in the bilayer system from platelet lipids would greatly enhance the studies on clotting factor-lipid interactions.

Aminophospholipid labelling by TNBS. Extent of probe penetration into whole platelets, plasma membranes and lipid vesicles

The probe, TNBS, has been revealed as a good tool for demonstrating the asymmetry of the erythrocyte membrane, because its charged sulfonic groups do not penetrate into the hydrophobic core of the membranes [10,11]. This reagent forms derivatives with membranes components containing primary amine groups [10] and can thus potentially react with only two of the platelet phospholipids, PE and PS. In order to use TNBS, it is important to establish the conditions where the probe does not penetrate into the structures, because it can sometimes cross the cell membranes [16,19]. Thus, whole platelets, artificial and natural membranes were incubated with a molar TNBS/lipid phosphorus ratio of 35 at room temperature for different times. The chemical modification by TNBS is followed by the loss of lipid phosphorus from the PE or PS spots and its appearance in the trinitrophenyl-PE and trinitrophenyl-PS spots. These results are shown in Fig. 1. The reaction is virtually complete after 5 min at room temperature and a maximum of 80 and 67% of the total PE and PS from solubilized lipids are trinitrophenylated, respectively. These values are stable after 12 h of incubation (unpublished data). In whole platelets, PS and PE are not available to TNBS during the initial 5 min, but in isolated plasma membranes or in artificial vesicles, 19 and 28% of total PE, and 17 and 13% of total PS are labelled after 5 min, respectively. An incomplete labelling of aminophospholipids with this probe has been observed in other materials [10,13,14,18]. Some authors explain this incomplete labelling on the basis of the strong interaction between lipids and proteins, by the introduction of bulky negatively charged

TABLE II

PHOSPHOLIPID COMPOSITION OF SHEEP PLATELETS AND THEIR SUBCELLULAR FRACTIONS

Phospholipids were separated by two-dimensional TLC. Results are expressed in terms of percent relative abundance within the four major phospholipids, determined as lipid phosphorus by the procedure of Bartlett [31]. The following unquantified phospholipids were detectable in trace amounts in all samples: cardiolipin, phosphatidic acid, PI, lysoPC and lysoPE. Data are the average (mean ± S.D.) of five experiments.

Fraction	Phospholipid species (%)			
	PC	PE	PS	sphingomyelin
Whole platelets	28.8 ± 1.3	37.5 ± 3.5	14.7 ± 2.2	17.6 ± 1.9
Plasma membranes (Bp)	28.6 ± 1.4	33.6 ± 2.4	12.7 ± 0.9	24.4 ± 1.5
Intracellular membrane + granules (Cp)	30.7 ± 3.5	36.5 ± 2.5	15.7 ± 1.7	19.4 ± 2.1
Granules (Dp)	34.0 ± 1.7	37.3 ± 2.7	11.9 ± 1.3	18.0 ± 1.2

TABLE III

PERCENTAGE DISTRIBUTION OF LIPID CLASSES IN SHEEP PLATELETS AND THEIR SUBCELLULAR FRACTIONS

Values are expressed as percentage of each lipid species (mean ± S.D.) obtained in four assays. Data are calculated by setting the total lipids (extracted in the lower organic phase of Folch, and determined gravimetrically) at 100%. The remainder are essentially considered as triacylglycerols, free fatty acids, diacylglycerols.

Component	Fraction (%)			
	whole platelet	plasma membrane (Bp)	intracellular membranes + granules (Cp)	granules (Dp)
PE	26.4 ± 1.8	22.7 ± 1.1	23.8 ± 1.4	26.1 ± 2.1
PC	20.9 ± 1.5	19.3 ± 0.2	20.0 ± 2.0	23.8 ± 2.2
PS	10.3 ± 0.8	8.6 ± 0.9	10.2 ± 1.4	8.3 ± 1.1
Sphingomyelin	12.4 ± 1.1	16.5 ± 0.4	12.6 ± 1.1	12.6 ± 0.4
Free cholesterol	24.3 ± 1.3	25.7 ± 1.5	25.2 ± 1.2	21.0 ± 1.1
Cholesteryl esters	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1
Remainder	5.2 ± 0.4	6.4 ± 0.5	9.1 ± 0.8	8.8 ± 0.8

groups on the surface. It is not difficult to envisage that the association of these headgroups causes restrictions to the lipid solutions or to the surface of membranes, hindering the reaction on its way to completion [18]. The lipid charge and their fatty acid composition are other rate-determining factors in the reactivity of TNBS with aminophospholipids [35].

Our results indicate that in intact platelets, the increase in incubation time with TNBS facilitates penetration of the probe, probably because the platelets are disrupted and the internal PE and PS molecules are exposed to TNBS. We can conclude that a reagent known to be nonpermeant [13], may penetrate, depending on the experimental conditions, as our results demonstrate.

Model for aminophospholipid asymmetry in platelet plasma membranes and in liposomes

The transbilayer distribution of PE and PS has been determined by the TNBS reaction. The interpretation of experimental data is based on the behaviour of the putative nonpermeant probe. TNBS does not diffuse across the bilayer into the interior of isolated plasma membranes and lipid vesicles, even after 120 min of incubation with the probe. Nevertheless, when the incubation time of intact platelets with TNBS is increased, the probe penetrates into the platelets, but permeation is imperceptible at 15 min (see Fig. 1). Accordingly, the data are given at 15 min of treatment with TNBS, because at this time we are sure that the

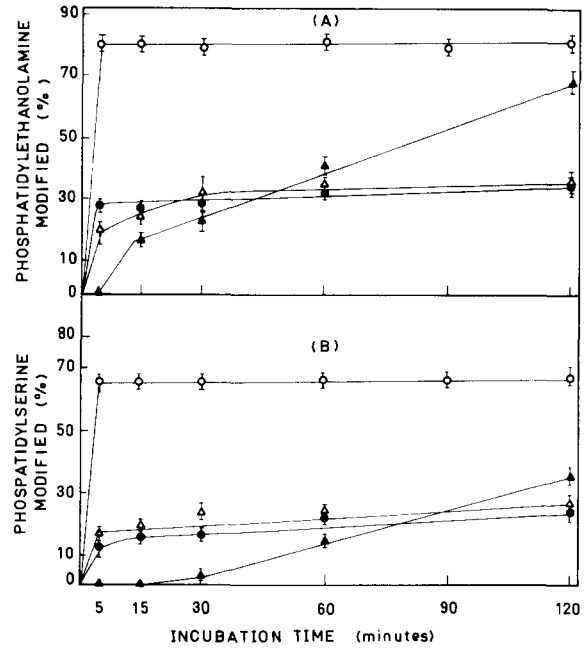

Fig. 1. Modification of PE (A) and PS (B) by TNBS in solubilized lipid extracted from platelets (○), whole platelets (▲), platelet plasma membrane (△) and liposomes from platelet lipids (●). Samples were incubated with a molar TNBS/lipid phosphorus ratio of 35 at room temperature and withdrawn at the indicated times. The reactions were stopped in acidic medium. The percentages of trinitrophenyl-PE and trinitrophenyl-PS were determined as described in the text (see Results and Discussion). (When symbols and error bar limits coincide, the latter have been omitted for clarity.)

aminophospholipid labelling reaction goes to completion in all systems.

A selected population of vesicles was obtained from total lipids extracted from whole platelets by the detergent-dialysis method [28]. Liposome size was 220 nm as average diamater (unpublished results), and the phospholipid-to-cholesterol molar ratio was 0.67. The real distribution of aminophospholipids in these liposomes was asymmetric, with about 23% of total PS ($15.2 \times 100/67$) and 34% of total PE ($27.1 \times 100/80$) present on the outer monolayer (see Table IV). This model has been postulated, considering that only 67% of total PS and 80% of total PE from solubilized lipids is trinitrophenylated and knowing the percentage of modification of PS and PE in liposomes after 15 min (data from Fig. 1). Similar values were obtained using isolated plasma membranes from whole platelets, followed by treatment with TNBS. The results show that about 28% of total PS ($18.9 \times 100/67$) and 31% of total PE ($24.8 \times 100/80$) are located on the external surface (see Table IV). In both of them, the PS and PE have similar orientations, with a preferential location inside the bilayer. However, Lee and Forte found that the PE-containing PC liposomes have about 56% of the total amino groups on their external surface, and conclude that the nature of asymmetric distribution of PE between the two monolayers of these small single-walled vesicles is the result of the combined properties of acyl side-chain constituents, and headgroup packing requirements of the phospholipids involved [19]. Vale demonstrates the preferential localization of PE on the outer and PS on the inner layer of the sarcoplasmic reticulum membrane from rabbit white muscle [18]. The distribution of phospholipids in various membranes from rat liver organelles is confusing, because each author provides a different answer [21,20].

In unstimulated and intact sheep platelets, PS is inaccessible to the probe. The real distribution of each aminophospholipid class in the outer leaflet of sheep platelet surfaces (a), has been calculated by the following expression: $a = A \cdot 100/x$, where A represents the total amount of an aminophospholipid modified by TNBS, and x is the percentage of each phospholipid located in the plasma membrane. The x values can be determined by using the data summarized in Table V. For instance, plasma membrane PE represents 0.617 (61.7/100) of the total amount present in the particulate fractions, whereas plasma membrane PS is about 0.653 (65.3/100). These data indicate that about 58–65% of each sheep platelet phospholipid would be located in the plasma membrane, and are in excellent agreement with those obtained by Chap et al. in pig platelets [23], and by Perret et al. in human platelets [24].

Taking this into account, it is concluded that 18% of plasma membrane PE is located in the outer-half of sheep platelet plasma membrane, whereas PS appears to be nearly exclusively located on the cytoplasmic surface. If it is considered that in phospholipids extracted from platelets and incubated with TNBS, only 80% PE is labelled after 15 min of incubation, then 22% of this aminophospholipid would be located in the external part of

TABLE IV

PERCENTAGE DISTRIBUTION OF AMINOPHOSPHOLIPIDS IN LIPOSOMES, ISOLATED PLASMA MEMBRANES AND SURFACE MEMBRANES FROM WHOLE PLATELETS

These distributions represent the greatest theoretical percentage of aminophospholipids on the outer leaflet of the bilayer, and the smaller theoretical percentage on the cytoplasmic leaflet (for details of the calculation, see Results and Discussion).

Component	Fraction (%)					
	Liposome		Isolated plasma membrane		Whole platelet	
	external leaflet	cytoplasmic leaflet	external leaflet	cytoplasmic leaflet	external leaflet	cytoplasmic leaflet
Aminophospholipids	30.5	69.5	30.2	69.8	15.8	84.2
PE	33.9	66.1	31	69	22	78
PS	22.7	77.3	28.2	71.8	0	100

TABLE V

PERCENTAGE DISTRIBUTION OF PHOSPHOLIPID SPECIES IN PARTICULATE FRACTIONS FROM SHEEP PLATELETS

Results are expressed as percentage of total lipid phosphorus (mean ± S.D. of four assays) by assuming that lipid phosphorus of each phospholipid class is 100%.

Particulate fraction	Phospholipid species (%)			
	PC	PE	PS	sphingomyelin
Plasma membrane (Bp)	58.1 ± 2.0	61.7 ± 1.6	65.3 ± 2.3	63.8 ± 1.6
Intracellular membrane + granules (Cp)	14.4 ± 1.0	14.9 ± 0.5	12.7 ± 0.6	12.8 ± 1.7
Granules (Dp)	28.6 ± 1.6	23.2 ± 2.3	21.6 ± 2.4	23.5 ± 2.2

plasma membrane (Table IV). These results confirm those obtained by Schick et al. in human platelets [13], using the same chemical probe.

This nonrandom phospholipid distribution of blood cell surface membrane may serve for a biological purpose: it may contribute to maintaining the delicate balance between regulating hemostasis and avoiding thrombosis [1,9], by the presence of procoagulant phospholipids, preferentially in the cytoplasmic-half of blood cell membranes. The precoagulant phospholipids are anionic compounds (PS and PI), and they exert a powerful surface catalytic action on the plasma-coagulation proteins, as has been demonstrated by several authors [1,9,36]. Changes in their distribution during platelet activation have been reported [13,37].

From our results, we conclude that there is a preferential location of aminophospholipids on the inner surface of liposomes and cell surfaces of sheep platelets. This distribution is consistent with previous data reported by other authors in platelets [23,24] and erythrocytes from various mammalian species [10,12], and with the physiological role played by anionic phospholipids in blood coagulation [9].

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