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The activation of rat platelets increases the exposure of polyunsaturated fatty acid enriched phospholipids on the external leaflet of the plasma membrane

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Rat platelets have been hydrogenated in the presence of colloidal palladium adsorbed on the surface of the non water-soluble polymer polyvinylpolypyrrolidone. This non-permeating catalyst restricts hydrogenation of the fatty acyl double bonds of phospholipids only in the outer half of the plasma membrane. The pattern of hydrogenation of the molecular species present on the external side of the membrane is determined using desorption-chemical soft ionization-mass spectrometry (DCI-MS) before and after cell activation by the calcium ionophore A23187. The accessibility to the catalyst of the polyunsatured molecular species within each phospholipid class is compared for resting and activated cells. The abundance of polyunsaturated species of phosphatidyl-ethanolamine and -serine in the inner half of the resting biomembrane is confirmed in rat platelets. Phosphatidyl-choline is especially rich in disaturated species in this membrane. The induced exposure of the polyunsaturated species of diacyl- and ether-phosphatidylethanolamine, and of phosphatidylserine on the external side of the membrane appears after activation by the calcium ionophore. A detailed quantitative analysis within a phospholipid class shows an unequal scrambling for diacyl-, alkyl-, alkenyl-phosphatidylethanolamine, and a variable involvement in the transmembrane redistribution following cell activation of the various molecular species as a function of the acyl moities.

Introduction

The disappearance of the phospholipid asymmetric distribution after cell activation is illustrated by exposure of polar headgroups localized in the internal leaflet of the resting cells to non-permeating external modifiers (phospholipases or chemicals targeted toward the amino group). The inside-outside distribution of PE and PS has been reviewed [1] in activated or resting platelets. The susceptibility of these phospholipids to sphingomyelinase and to phospholipase A_2 in the activated platelets becomes increased after the extensive secretion of the granular content induced by calcium ionophore [1]. The scrambling could be a consequence of the multiple fusions occurring between the internal granules and the cytoplasmic membrane leading to secretion. The relationships between the topological change and the Ca^{2+} influx, the extent of the exocytosis and lysis, the opposite polarities for granules and plasmic membranes to undergo fusion, or the transient inhibition of the aminophospholipid translocase [2] have still to be clarified for the range of aggregating agents which show different influences. Ionophore or diamide give an extensive scrambling whereas thrombin gives a limited effect. Transient non-bilayer structures as a result of diglyceride production by phospholipase C [3] or as a result of a Ca^{2+}-calpain induced alteration of the cytoskeleton [4] are proposed causes for the loss of the lipid asymmetry either preceding or following membrane fusion.

Because changes in the membrane topology of activated cells are relatively fast, they are assumed to result from the redistribution of whole phospholipid molecules without cleavage of the polar headgroup from the fatty acyl moities. During the short activation time, the extent to which the molecular species of native PL are altered by stimulated phospholipases and transacylase activities [5], is questionable. If these enzymatic alterations were to be taken into account, a fatty

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Abbreviations: PL, phospholipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; DCI-MS, desorption chemical ionization–mass spectrometry; TNBS, trinitrobenzene sulfonic acid.

acid redistribution would be superimposed to the scrambling of the phospholipid classes.

The highly unsaturated chains of PS and PE are assumed to fluidize the outer half of the plasma membrane in activated platelets. This is consistent with an increased binding of the packing-sensitive dye, merocyanine [6]. This could be of a physiological consequence if it favours the diffusion-limited interaction between the surface activated coagulation proteins forming the tenase ($IX_a + VIII_a$) and the prothrombinase ($X_a + V_a$) complex.

The asymmetric distribution of the different molecular species within a membrane phospholipid class has been debated for the red blood cell [7] and for some other cell types. A non-random distribution is suggested for PE [7], etherPE [8], and SM [9]. A difference is observed between nucleated cells where the cytofacial leaflet is more rigid compared with red cells where it is more fluid [10]. Differences have been noted between human and rat red cell membranes for the molecular species of PE [7]. An influence of the diet on polyunsaturated fatty acids distribution has been studied for these red blood cells [7].

The non-random distribution of the different molecular species has been presumably associated with an acyl chain specificity of the aminophospholipid translocase [11] or with a differential 'physical' flip-flop rate [12], the polyunsaturated species showing a faster transversal diffusion.

To study the fatty acyl distribution in the rat platelet membranes we take advantage of the high activity of a non-permeating, non-toxic, surface hydrogenation catalyst [13]. This polymer-anchored colloidal palladium derivative (Pd sols) was demonstrated to be active upon the thylakoid membrane of *Synechocystis* cyanobacteria. Preliminary studies with human platelets have also established that the catalyst is able to alter the fluidity of the external leaflet of the membrane probed by TMA-DPH [14].

In the present work, the catatalyst was used to probe the location of lipid molecular species in the platelet membrane and observe changes upon activation.

Materials and Methods

Fresh rat platelets are prepared by differential centrifuguation [15]. The platelets are suspended in 2 ml gelatin-enriched (0.25%) Tyrode buffer (approx. 10^9 cells/ml) for aggregation by Ca ionophore A23187 (1.25 μM). The aggregation is controlled by light scattering under appropriate conditions of stirring and temperature (Aggrometer Chronolog Coultronic). After 120 s, the activated cells are centrifuged, collected and resuspended in 2 ml saline phosphate buffer (pH 7) to be added to the pre-activated catalyst suspension (prepared as described below). The gas phase is purged and replaced with H_2 (or N_2 in control experiment) several times, and the sample is hydrogenated for 30 min at room temperature under stirring.

Hydrogenation of platelets is achieved under 1 atm of pure H_2 at room temperature in a Schlenk-type tube of 10 ml wherein a disposable 5-ml plastic tube is placed with a small magnetic bar. To prepare the catalyst Pd sols suspension in this tube, 100 mg insoluble cross-linked polyvinylpolypyrrolidone (PVPP Plasdone XL from Fluka) and 10 mg $(NH_4)_2PdCl_4$ (from Aldrich) are previously mixed in 2 ml phosphate buffer (pH 7) for 10 min. Then, the Schlenk tube is sealed with a stopper fitted with a silicone rubber septum. The Schlenk-tube is purged repeatedly and filled with H_2. Palladium black starts to precipitate immediately and the mixture is kept stirring under H_2 for 20 min. After the suspension is let to settle, the supernatant is removed using a needle through the septum and the platelets are introduced.

To ensure that the non permeant catalyst could not reduce double bonds of phospholipids belonging to the internal side of the membrane, sonicated dioleoyllecithin vesicles had been treated by the Pd-sols catalyst in a preliminary test. The experiment, under the conditions described for treatment of the platelets, showed that after 3 min, the conversion of oleic acid to stearic acid is completed and leveled off at 60%. The level of the conversion remained unchanged during at least 30 min. This maximum value would represent the dioleoyllecithin forming the outer monolayer of the highly curved vesicle membranes.

After hydrogenation, the platelet lipids are extracted from the hydrogenation mixture by a procedure adapted from Bligh [16]. The washing of the lower chloroform phase is completed by 3 volumes of fresh upper hydro-alcoholic phase, then dried on anhydrous Na_2SO_4 and concentrated before being spotted down on the TLC plate.

The separation of the PL classes is obtained by eluting the TLC plate (Silica Gel F1500 from Schleicher and Schuell, Germany) in the solvent system chloroform/methanol/isopropanol/aqueous KCl 0.25%/triethylamine (30:9:25:6:18, v/v) as described in Ref. 17. This solvent system has been selected for an efficient resolution of the various platelet phospholipids with a special emphasis on PS. After spraying with fluorescein, the spots detected under UV are scrapped off and extracted by chloroform/methanol (1:1, v/v).

The phospholipid spots are identified by MS and the molecular species (acyl chains carbon number: double bonds number) corresponding to the various fatty acyl combinations are quantified. The MS method is Chemical Ionization by ammonia as a reagent gas (10^{-1} Torr) and Direct Introduction-Thermal Desorp-

tion (DCI) of the sample in the source (Nermag R10-10C mass spectrometer). The soft ionization procedure has been adapted from Refs. 18 and 19. The positive ions are detected in the mass range 450–850 within a scan time of 2 s and the thermal desorption current rise is 10 mA/s.

The quantification of the different molecular species by time-integration of the different mass peaks present in the successive spectra has been challenged with a procedure of HPLC [20,21] which quantifies the UV absorbance of benzoylated derivatives of the diglycerides obtained from the various platelet phospholipids after phospholipase C hydrolysis.

To detect the activation of platelets under our experimental conditions (especially during the hydrogenation procedure), the release of radiolabeled 5-hydroxy[2-^{14}C]tryptamine (creatinine sulfate salt, 1.85 GBq/mmol (Amersham)) from prelabeled cells is measured. Platelets are pre-labeled after incubation of the platelet-rich plasma (6 ml) with radioactive serotonin (6 μl) for 45 min. The percentage of released serotonin is compared with resting or ionophore-activated control platelets.

Results

(I) Formation of the mass spectra in the DCI mode

In Fig. 1 the upper pattern is the mass spectrum of platelet PE after activation and hydrogenation by the non-permeating catalyst. The lower pattern is for unactivated platelets which have been similarly hydrogenated. The lowest spectrum (quoted 'comparison') is a differential spectrum of (activated platelets) minus (unactivated platelets). It illustrates the difference appearing between the patterns of hydrogenation of PE under the influence of the cell activation. It is explained by the overexposure of PE molecular species brought about by the activation.

For each molecular species, two mass peaks are observed: (i) $M - 140$ emerges from the cleavage of the phosphorylethanolamine headgroup of the molecular ion and (ii) $(M + 35) - 140$ from the same cleavage of the quasi-molecular ion formed by an adduct $(+35)$ with the reagent gas, $(NH_3)_2H^+$. The ratio $M - 140/(M + 35) - 140$ varies as a function of the temperature and of the pressure of ammonia in the source.

For example, the major 18:0/20:4 molecular

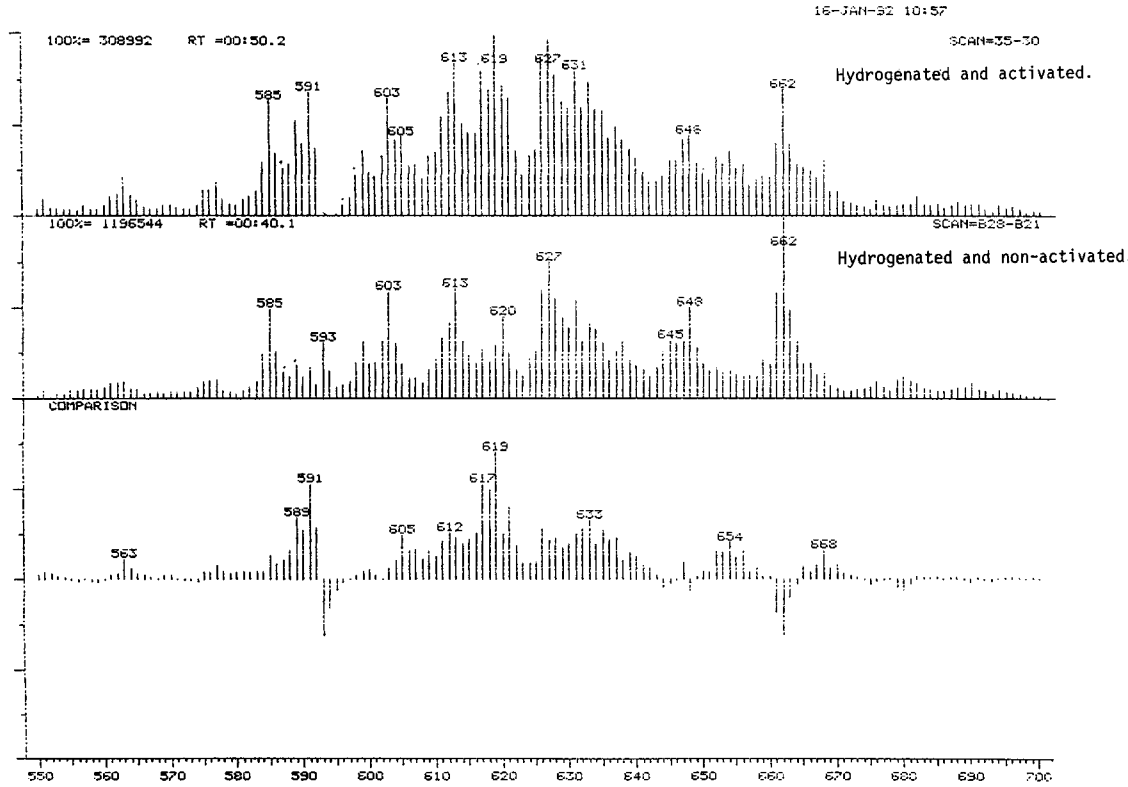

Fig. 1. DCI (ammonia) mass spectra of phosphatidylethanolamine molecular species from rat platelets activated by ionophore A23187, 1.25 μM (upper spectrum) or unactivated (lower). Both samples of platelets have been hydrogenated by the non-permeating catalyst for 30 min. The spectrum quoted 'comparison' is the difference (upper spectrum = activated) − (lower spectrum = unactivated). The correspondance between the mass peaks and the corresponding molecular species of diacylphospholipids (carbon number:double bonds) are quoted in Fig. 5 (for $M + 35$) or Fig. 7 (for M) along the horizontal axis. The correspondance for ether phospholipids is shown in Fig. 8.

species of PE (MW-767) releases two proeminent mass peaks in the spectrum, 662 ($M + 35 - 140$) and 627 ($M - 140$). The 'comparison' spectrum displays the reduction of this polyunsaturated PE in ionophore-activated platelets, which gives rise to an increase of ions 664, 666, 668, 670 from 662, and 629, 631, 633, 635 from 627.

The assignment to a mass of a particular molecular formula is in agreement with the fatty acid composition determined previously by GLC and by HPLC [20,21]. The qualitative and quantitative agreement between the methods was challenged for platelets diacylPC (10 HPLC determinations compared with 3 MS determinations). Discrepancies are noted for the minor species which are not taken into account on the HPLC pattern because of a lack of sensitivity. On the contrary, some species are probably overestimated by MS due to ^{13}C isotopic abundance and to multiple proton rearrangements. HPLC does not allow resolution between some molecular species (for example, 18:0/18:2 and 16:0/18:1). Consequently the number of different absorption peaks (= 11) considered in HPLC patterns is lower than the number of resolved mass peaks (= 19) by MS.

Because rat platelet PE contains, respectively, 44% and 29%, as alkenyl and alkyl derivatives [20], ether phospholipids have to be separated from the diester before HPLC. This step is unnecessary for MS analysis because the corresponding mass peaks (respectively, −14 and −16 for alkyl- and alkenyl- relative to diacyl-PE) can be assessed directly in the mass spectrum. The proportion of the different classes (diacyl, alkyl, and alkenyl) are biased because of a different cleavage of the vinyl ether ions. However, for one type of chemical bond considered at the 1 position, the molecular species pattern remains reliable. In Fig. 1, three proeminent etherPE mass peaks, 585, 611, and 613, have been assigned, respectively, to 16:0/20:4 alkylPE, 18:0/20:4 alkenylPE and 18:0/20:4 alkylPE.

Fig. 1 shows the gradual saturation of the fatty acyl double-bonds as a result of the activity of the catalyst: for instance, diacylPE 18:0/20:4 (662, 627) give rise after hydrogenation of 1, 2, 3 or 4 double-bonds to diacylPE 38:3 (664, 629), 38:2 (666, 631), 38:1 (668, 633) and 38:0 (670, 635). The reduction of three double-bonds of the arachidonyl moiety to 1 double-bond (to the mass 633 and 668) is the most frequent occurrence as noticed in previous kinetic studies with heterogeneous catalysts [22] acting for a limited time (presently 30 min).

Ether derivatives of PE are reduced along with the diacyl. For instance, 18:0/20:4 alkylPE and alkenylPE are reduced to the mono-unsaturated derivatives (see Fig. 8). Hydrogenation byproducts from alkyl- and from alkenyl-etherPL cannot be quantitated accurately because their parent compounds differ by only 2 atomic

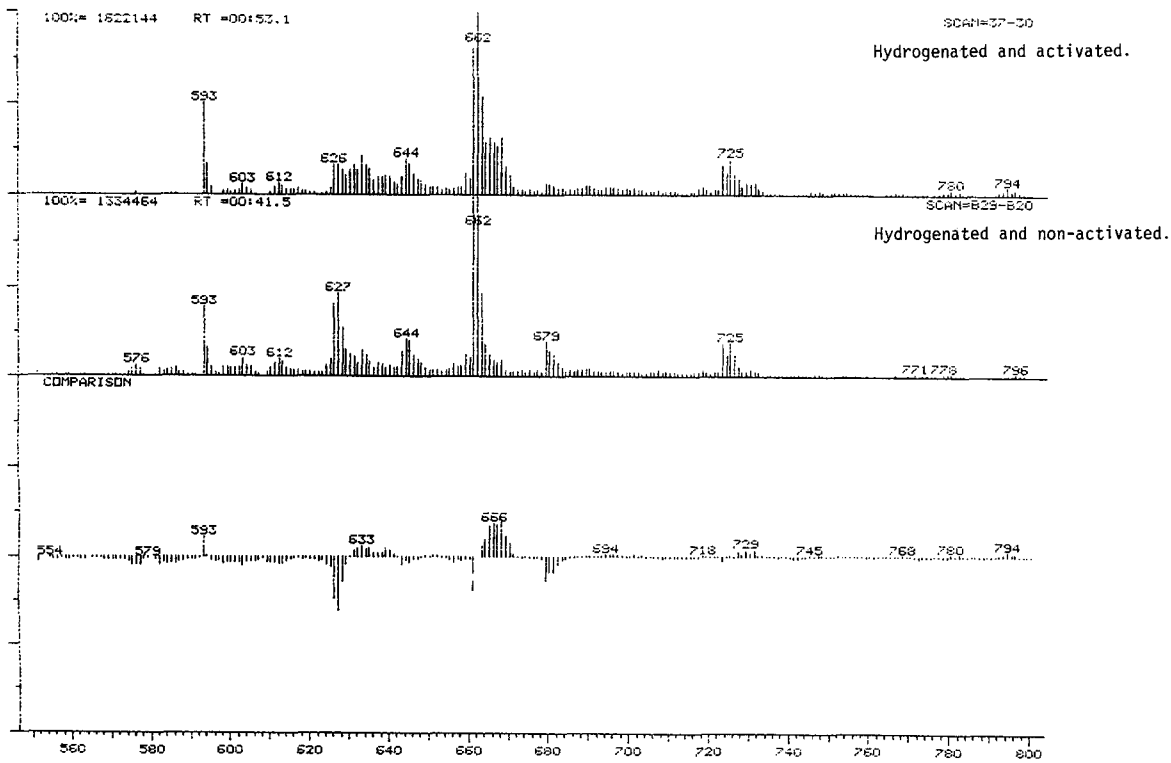

Fig. 2. DCI (ammonia) mass spectra of phosphatidylserine molecular species from rat platelets activated by ionophore (upper spectrum) or unactivated (lower). The platelets have been hydrogenated by the non-permeating catalyst for 30 min before PS separation on TLC. The spectrum quoted 'comparison' is the difference (upper spectrum = activated) − (lower spectrum = unactivated).

mass units. This results in a significant crossed-'contamination' of the alkyl from the alkenyl mass peak due to ^{13}C abundance (for a 50 carbons molecule, $(M+2)/M = 30\%$).

Neither the nature of the chemical bond of the fatty chain with the glycerol backbone, nor the length of the fatty chain at the 1-position are selective as regard to the activity of the catalyst [22]. For instance, the linoleoyl chain in 18:0/18:2 (603) is reduced to the mono-unsaturated derivative (605).

The position relatively to the membrane interface is influential on the hydrogenation rate. Previous studies [22] with heterogeneous catalysts acting on control lecithins have shown that the rate of reduction follows the order: arachidonic ≫ oleic. This order parallels the number of $-CH_2-$ between the carbonyl group and the first double bond accessible to the catalyst acting from the lipid/water interface ($\Delta 5$, $\Delta 9$). 30 min hydrogenation time (the present experimental conditions) ensures that every accessible polyunsaturated phospholipid on the outer monolayer of the membrane will be reduced by the catalyst [22] in spite of the difference of susceptibility among the various polyunsaturated species. The rate of conversion differs according to the double bond position within the membrane depth but the conversion of polyunsaturated chains to monounsaturated or saturated chains will be completed within this relatively long time. To establish an index informative of the lipid topology between the hydrogenation pattern and the exposure to the catalyst, only the decrease of the most unsaturated species is taken into account. This index is not ambiguous because it does not deal with the numerous byproducts of the hydrogenation that cannot be related to a unique parent compound and it is not influenced by the degree of completion of the hydrogenation. After 30 min hydrogenation, the mono-unsatured species (i.e., 18:1 or 20:1) result from the difference between the rate of reduction from polyunsaturated species and the hydrogenation to fully saturated species. Only the most unsaturated derivatives decrease as a simple function of the catalyst activity, other derivatives formed after hydrogenation being substrates as well as byproducts of this activity.

A qualitative comparison of mass spectra of PE and PS (Figs. 1 and 2) derived from unactivated and from activated platelets confirms a variable exposure to the PVPP-anchored catalyst. The exposure of the most unsaturated molecular species to the catalyst is estimated by a decrease of the corresponding mass peak. The decrease between non-hydrogenated resting platelets and hydrogenated resting platelets is related to the accessibility of this molecular species in the resting native membrane. The decrease between resting hydrogenated platelets and activated hydrogenated platelets gives an insight on the additional exposure following the cell activation. The pattern of PC is

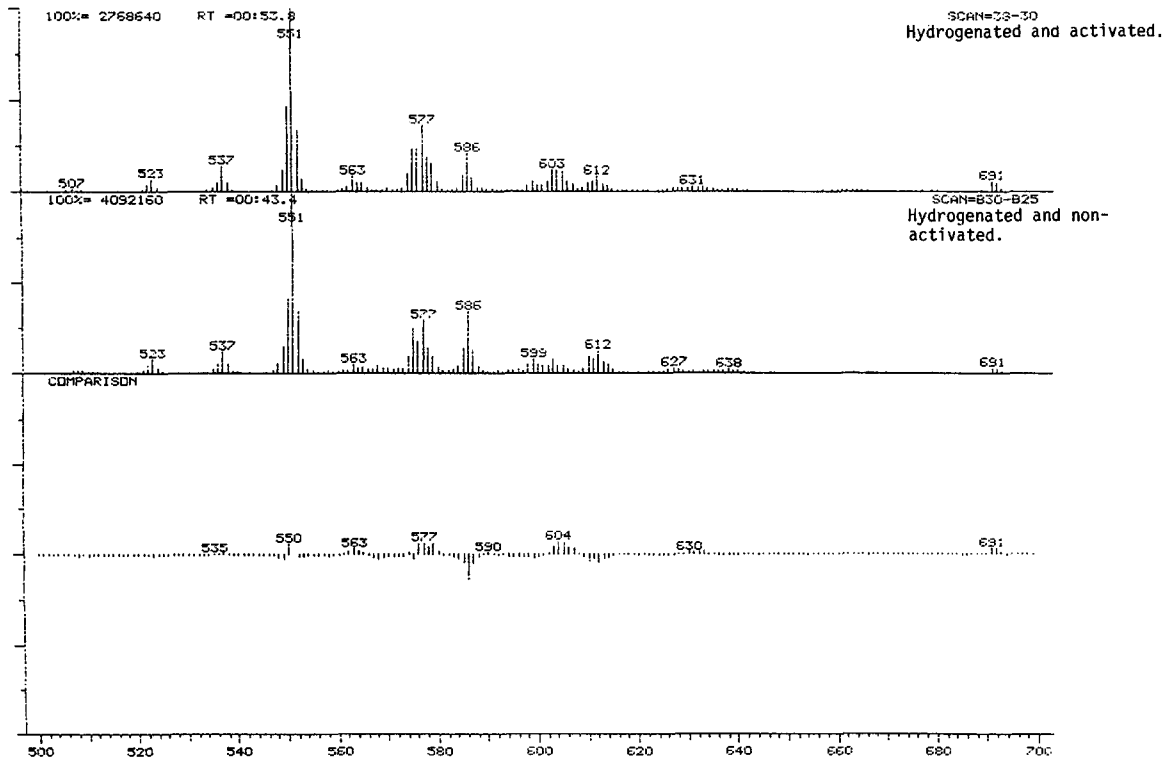

Fig. 3. DCI (ammonia) mass spectra of phosphatidylcholine molecular species from rat platelets activated by ionophore (upper spectrum) or unactivated (lower). The platelets have been hydrogenated by the non-permeating catalyst for 30 min before PC separation on TLC. The spectrum quoted 'comparison' is the difference (upper spectrum = activated) − (lower spectrum = unactivated).

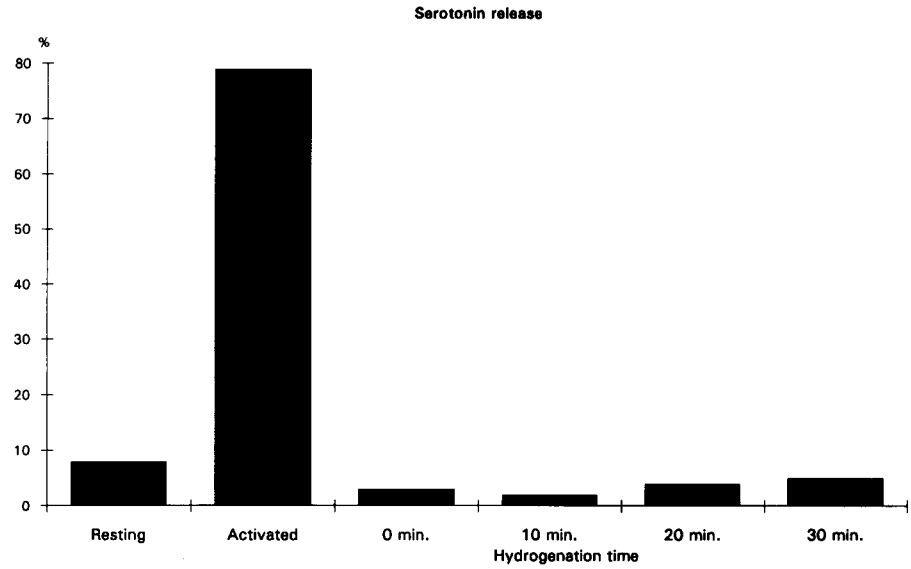

Fig. 4. Release of radioactive 5-hydroxy[2-^{14}C]tryptamine from prelabeled platelets during the hydrogenation procedure. Platelets are hydrogenated for various incubation times at room temperature in the presence of 100 mg Pd sols catalyst. At intervals, an aliquot is sampled, spun down and the supernatant radioactivity is counted. In the positive control, the cells are activated by ionophore 1.25 μM.

slightly altered after stimulation (Fig. 3). PC in rat platelets was recognized for its high content in disaturated species on the basis of analysis by HPLC [20] (approx. 40% 16:0/16:0 diacylPC (586 and 551)). The lack of sensitivity of this species to the catalyst prevents any conclusions with regard to its accessibility. A similar situation is observed for the highly saturated sphingomyelin fraction (data not shown).

The long hydrogenation time (30 min) could have been damaging to the cells. Of particular concern is the possibility of platelet activation and penetration of the catalyst inside the disrupted cells. Fig. 4 presents the results of serotonin release as a monitor of cell activation and lysis. Lenghtening hydrogenation time to 30 min does not increase the release of the granule marker (1.9–4.6%) compared with non-activated control (7–9%) platelets. The release is negligible in the saline phosphate buffer (pH 7) used during the hydrogenation step as compared to ionophore activated cells (release = 79%). The insoluble Pd-sols under a nitro-

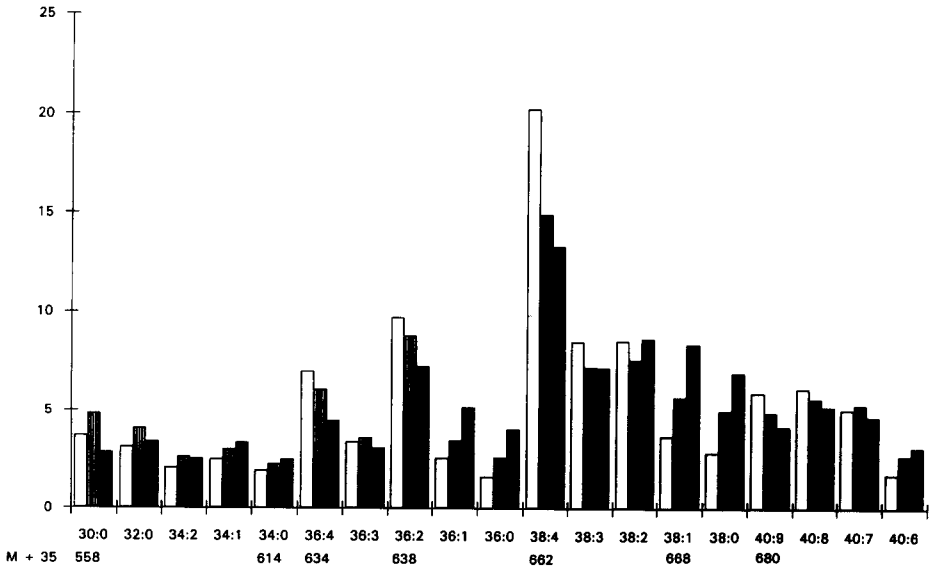

Fig. 5. Relative amounts of the molecular species of diacylphosphatidylethanolamine in unactivated rat platelets before (□) or after reduction (▥) by the non-permeating hydrogenation catalyst and in platelets activated (■) by the ionophore A23187 (1.25 μM). The mass axis is expressed as carbon number: double-bonds of both fatty acyl moities. The percentages refer to the sum of the mass peaks selected from the spectra for integration. The standard deviations ($n = 3$) after hydrogenation are, respectively, 0.62, 0.26, 0.39% for 38:4, 36:4 and 40:8 before activation and 0.93, 0.21 and 0.33% after activation by the ionophore.

TABLE I

Relative accessibility of non-activated and activated cells

Accessibility is calculated for non-activated and activated cells relative to the native non-hydrogenated state. Overexposure is the increase in accessibility following the activation by ionophore. n.d., not determined.

		DiacylPE		AlkenylPE (583 & 611)		AlkylPE (585 & 613)		DiacylPS	
		access.	overexp.	access.	overexp.	access.	overexp.	access.	overexp.
16:0/20:4	non-activated	13%	23%	n.d.	n.d.	38%	23%	n.d.	n.d.
	activated	36%		n.d.		61%		n.d.	
18:0/20:4	non-activated	26%	8%	33%	14%	37%	10%	45%	19%
	activated	34%		47%		48%		63%	

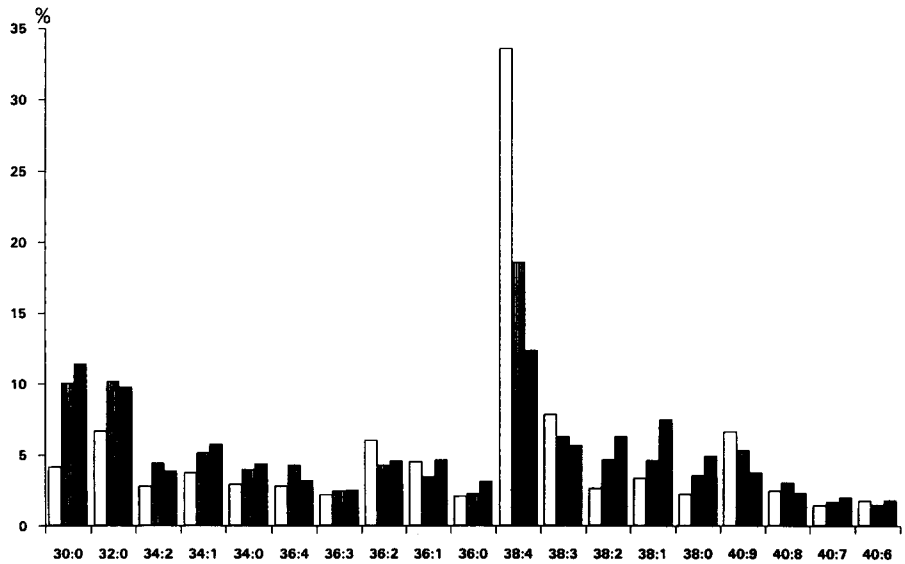

Fig. 6. Relative amounts of the molecular species of diacylphosphatidylserine before (□) and after reduction (▥) by the non-permeating hydrogenation catalyst in rat platelets unactivated or activated (■) by ionophore A23187 (1.25 μM). The standard deviations ($n = 3$) after hydrogenation are, respectively, 0.95, 0.49 and 0.52% for 38:4, 36:4 and 40:8 before activation and 0.97, 0.21 and 0.49% after activation.

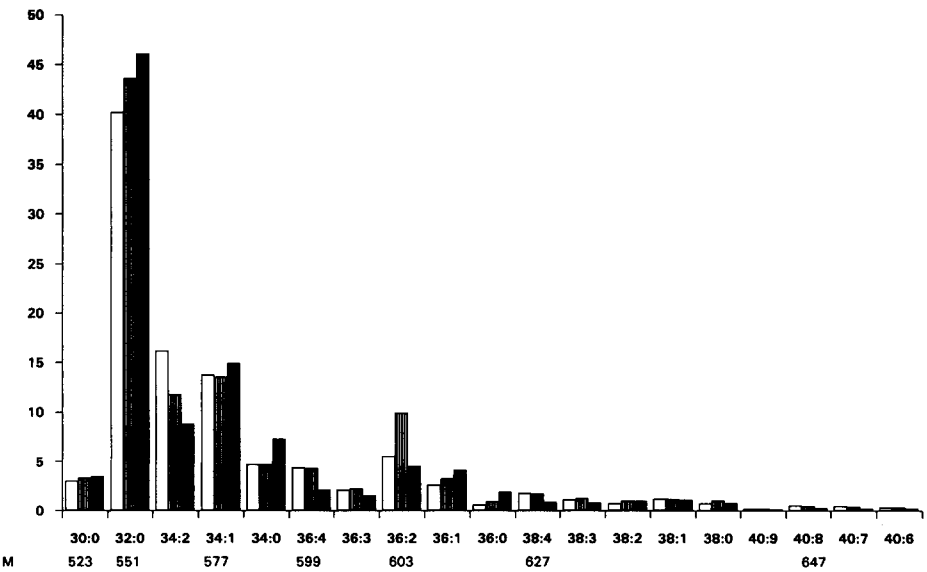

Fig. 7. Relative amounts of the molecular species of diacylphosphatidylcholine before and after reduction by the non-permeating hydrogenation catalyst in rat platelets unactivated or activated by ionophore A23187 (1.25 μM).

gen atmosphere had been shown to have only a limited influence on the thrombin-induced aggregation of rat platelets (Joo, F., personal communication).

(II) Quantitative hydrogenation in resting or activated platelets

Because volatilization of the phospholipid in the ionization source requires longer than a scan-time (2 s) used to acquire one mass spectrum, quantitation must be performed by time-integration of the different masses throughout the thermogram of the phospholipid. The results of these computations are illustrated as histograms in the following figures.

PE and PS (Figs. 5 and 6) are rich in polyunsaturated species (especially 16:0/20:4, 18:0/18:2 and 18:0/20:4 for PE, 18:0/20:4 for PS) which become over-exposed to the activity of the external catalyst upon activation.

In diacylPE (Fig. 5 and Table I) the accessibility to the catalyst in unactivated platelets for 18:0/20:4 is 26.3%. It is lower for 16:0/20:4 (13%) but the over-exposure of 18:0/20:4 after activation is lower 8% versus 23% for 16:0/20:4. The final exposure for both species is 34–36%. In PS (Fig. 6 and Table I) the original exposure of 18:0/20:4 (44.6%) is much higher than in PE, and represents nearly 16% of the total PS. After activation an additional large (19%) increase of accessibility is produced. Finally 63% of 18:0/20:4 diacylPS become accessible to the catalyst after activation. Changes in some minor molecular species are observed in native diacylPC (Fig. 7) where 16:0/20:4 and 18:0/20:4 represent 4.4 and 1.8% of the overall molecular species. These polyunsaturated molecular species do not react with the catalyst in the resting cells, but after platelets have been challenged by the ionophore, these polyunsaturated molecular species become extensively reduced (respectively, −50 and −47%).

Subtle differences are observed for various molecular species within the same phospholipid class. For example, within alkylPE (Table I), both 16:0/20:4 alkylPE (585) and 18:0/20:4 alkylPE (613) are originally exposed by approx. 38%. After activation, their respective exposures increase by 23% and 10%. This illustrates a selective scrambling during the process of activation for two closely related molecules. The increased exposure of 18:0/20:4 alkenylPE (611) is +14%. Finally, in activated platelets, approximately one-third of polyunsaturated diacylPE, half of etherPE and two-third of diacylPS are exposed on the external surface of the membrane.

The hydrogenation in resting cells for 20:4-containing species is 28% in diacylPS, 14% in diacylPE and 4% in diacylPC relatively to native platelets. It reaches, respectively, 48%, 24%, and 51% after platelet activation by the calcium ionophore.

Discussion

The present data using the non-permeating palladium derivative as an hydrogenation catalyst to 'label' fatty acyl double bonds in the membrane illustrate one of the consequences of the PL scrambling during platelet activation. Other established methods using a non-permeable probe, such as trinitrobenzene sulfonic acid (TNBS) [23], or as a combination of phospholipases [25], provided a view focussed on the transverse distribution of the phospholipid classes. The present method discriminates between the different molecular species within a PL class.

The TNBS method applied to thrombin-activated human platelets [23] shows a 2-fold greater exposure (final 25%) of PE compared to unactivated platelets. TNBS labeled PS was not detected in human [23] or sheep resting platelets [24]. A phospholipid exchange protein method [30] has shown that arachidonyl-containing PS (a major species) behaves as a non-exchangeable pool in arachidonate radiolabeled human platelets. By contrast, the prothrombinase activation method or the susceptibility to phospholipases detect the presence of the acidic phospholipid on the platelet surface. The phospholipase method reveals a low and significant percentage of PS (6% in pig unactivated platelets, 9% in human platelets) on the outer monolayer [28,29]. This discrepancy illustrates presumably the repulsion of the anionic probe TNBS by acidic polar head groups arranged in the outer monolayer of the biomembrane in situ. The presence of PS on the outer half of the resting cell membrane may suggest the artefactual activation of platelets by phospholipases or in the presence of the Pd catalyst. In the present study the exposure of more than 16% of the cell PS to the external catalyst is noticeable. The absence of serotonin release supports the non activation of the platelets during the hydrogenation procedure. The nature of the buffer (pH 7), the absence of added calcium, and probably the PVPP granules hinder the trigger leading to platelets activation. Whether this amount of anionic phospholipid on the outer surface of the platelet is specific to rat platelets is also questionable. We suggest that TNBS or phospholipase method minimize the assay of the anionic phospholipid whereas the positively charged catalyst Pd sols is attracted by negatively charged polar head groups. The possibility that a fast turn-over of PS on the outside of the biomembrane could increase the final hydrogenation level, is unlikely on the basis that arachidonylPS is not found exchangeable following the phospholipid exchange protein method [30].

Because phospholipids (in particular, molecular species containing 20:4) are substrates for various enzymes (for instance, phospholipases and transacylase), the depletion in polyunsaturated phospholipids after

activation could be a priori the result of metabolic causes. But a very low release of 20:4 was previously demonstrated in rat platelets (10-fold less 20:4 released in rat compared with human platelets [26]). Only 2.5% of the total arachidonate of rat platelet PL are released [26] after activation.

Model membranes [12] and experimental data suggest that the fatty acyl groups could participate in the sideness of phospholipids resulting from an flip-flop equilibrium which favours the faster transverse diffusion of unsaturated species. The selective affinity of the aminophospholipid translocase [2] would alter the turn-over of unsaturated species of PE and PS between the two halves.

A difference of viscosity between the two halves results from the unequivalence of their acyl chain composition. The effect of the scrambling at the time of cell activation would be of significant biological relevance to make fluid the platelet surface in the coagulation cascade as discussed recently [1,2]. The propensity to form a non-bilayer arrangement, to fuse membranes together, to accommodate cholesterol, drug and proteins and to allow the lateral diffusion of the extrinsic proteins are consequences related to the changing asymmetric distribution of fatty acyl chains.

Assuming 30% of the cell PE on the outer half [28,29] an homogeneous distribution of every molecular species should release approx. 30% accessibility to the external catalyst. Much less is recorded in the resting cell for 36:4 or 36:2 diacylPE (respectively, 13 and 9%), and both species should be considered as internally-oriented. For etherPE an homogeneous distribution seems to be fulfilled for 36:4 and 38:4 alkylPE (38% and 37%) and for 38:4 alkenylPE (33%) (Fig. 8).

Mechanisms which selectively concentrate a molecular species within one PL class are to be clarified. The present data suggest the occurrence in the plasma membrane of resting rat platelets of such an asymmetrical distribution of the molecular species within PC, PE and PS. This asymmetric distribution is altered but not completely averaged out after activation. Some molecular species are slightly over-exposed whereas other species become extensively over-exposed upon the activation by the ionophore. The modulation of the scrambling of the phospholipid asymmetry could be oriented by the acyl chain moieties.

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References

1 Schroit, A.J. and R.F.A. Zwaal (1991) Biochim. Biophys. Acta 1071, 313–329.
2 Devaux, P.F. (1991) Biochemistry 30, 1163–1173.
3 Siegel, D., Banschbach, J., Alford, D., Ellens, H., Lis, L., Quinn, P.J., Yeagle, P.L. and Bentz, J. (1989) Biochemistry 28, 3703–3709.
4 Middelkoop, E., Van der Hoek, E.E., Bevers, E.M., Comfurius,

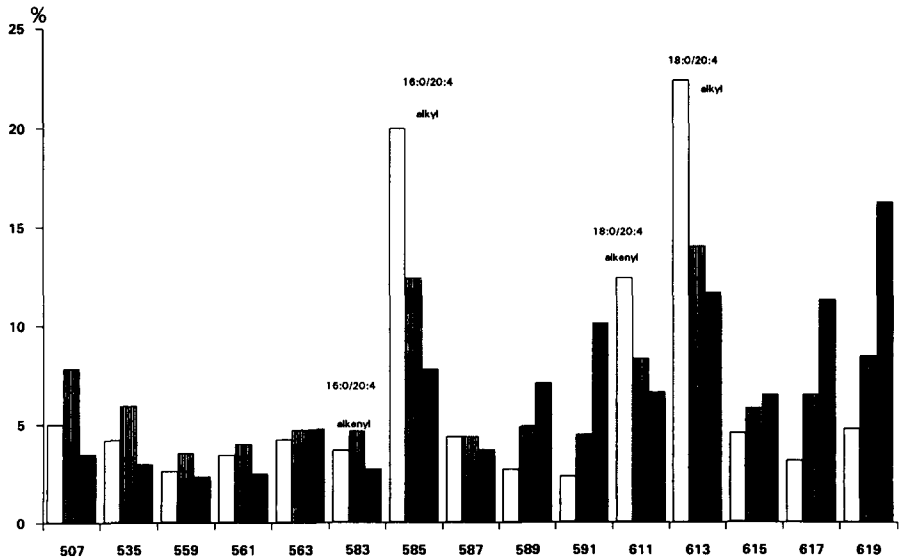

Fig. 8. Molecular species of etherPE (alkyl and alkenyl) before and after reduction by the non-permeating hydrogenation catalyst in rat platelets unactivated or activated by ionophore A23187 (1.25 μM). The mass are quoted and the major species are indicated in agreement with HPLC patterns of alkyl- and alkenyl-PE. The ions resulting from the vinyl ether lipid are comparatively lower than from the alkyl ether lipid due to an higher propensity for cleavage.

P., Slotboom, A.J., Op den Kamp, J.A.F., Lubin, B.H., Zwaal, R.F.A. and Roelofsen, B. (1989) Biochim. Biophys. Acta 981, 151–160.
5 Colard, O., Breton, M. and Béréziat, G. (1986) Biochem. J. 233, 691–695.
6 Schlegel, R.A. and Williamson, P. (1987) J. Cell. Physiol. 132, 381–384.
7 Hullin, F. and Salem, N. (1989) in Biomembranes and Nutrition (Léger, C.L. and Béréziat, G., eds.), Colloque INSERM, Vol. 195, pp. 77–86, Paris.
8 Record, M., El Tamer, A., Chap, H. and Douste-Blazy, L. (1984) Biochim. Biophys. Acta 778, 449–456.
9 Boegheim, J.P.J., Van Linde, M., Op den Kamp, J.A.F. and Roelofsen, B. (1983) Biochim. Biophys. Acta 735, 438–442.
10 Kier, A.B. and Schroeder, F. (1989) in Biomembranes and Nutrition (Léger, C.L. and Béréziat, G., eds.), Colloque INSERM, Vol. 195, pp. 67–76, Paris.
11 Morrot, G., Hervé, P., Zachowski, A., Fellmann, P. and Devaux, P.F. (1989) Biochemistry 28, 3456–3462.
12 Middelkoop, E., Lubin, B.H., Op den Kamp, J.A.F. and Roelofsen, B. (1986) Biochim. Biophys. Acta 855, 421–424.
13 Joo, F. and Vigh, L. (1991) in Catalytic Hydrogenation of Biological Membranes, Advances in Catalyst Design (Graziani, M. and Rao, C.N.R., eds.), pp. 254–266, World Scientific, Singapore.
14 Joo, F., Benko, S., Horvath, I., Torok, Z., Nadasdy, L. and Vigh, L. (1992) React. Kin. Catal. Lett. 48, 140–151.
15 Ardlie, M.G., Packman, M.A. and Mustard, F.J. (1970) Br. J. Haematol. 19, 7–17.
16 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Biophys. 37, 911–917.
17 Momchilova-Pankova, A.B., Markovska, T.T., Koshlukova, S.E. and Koumanov, K.S. (1991) J. Lipid Mediators 3, 215–223.
18 Bisseret, P., Nakatani, Y., Ourisson, G., Hueber, R. and Teller, G. (1983) Chem. Phys. Lipids 33, 383–392.
19 Ayanoglu, E., Wegmann, A., Pilet, O., Marbury, D., Hass, J.R. and Djerassi, C. (1984) J. Am. Chem. Soc. 106, 5246–5251.
20 Masrar, H., Béréziat, G. and Colard, O. (1990) Arch. Biochem. Biophys. 281, 116–123.
21 Joly, F., Breton, M., Wolf, C., Ninio, E. and Colard, O. (1992) Biochim. Biophys. Acta 1125, 305–312.
22 Quinn, P.J., Joo, F. and Vigh, L. (1989) Prog. Biophys. Mol. Biol. 53, 71–103.
23 Schick, P.K., Kurica, K.B. and Chacko, G.K. (1976) J. Clin. Invest. 57, 1221–1226.
24 Sanchez-Yagüe, J. and Llanillo, M. (1986) Biochim. Biophys. Acta 856, 193–201.
25 Bevers, E.M., Comfurius, P. and Zwaal, R.F.A. (1983) Biochim. Biophys. Acta 736, 57–66.
26 Masrar, H. and Colard, O. (1991), C.R. Soc. Biol. 185, 31–36.
27 Colard, O., Breton, M., Pepin, D., Chevy, F., Béréziat, G. and Polonovski, J. (1989) Biochem. J. 259, 333–339.
28 Chap, H.J., Zwaal, R.F.A. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 467, 146–164.
29 Perret, B., Chap, H.J. and Douste-Blazy, L. (1979) Biochim. Biophys. Acta 556, 434–446.
30 Chambaz, J., Wolf, C., Pépin, D. and Béréziat, G. (1980) Biol. Cell. 37, 223–230.