ELSEVIER

Biochimica et Biophysica Acta 1235 (1995) 249–255

BBA Biochimica et Biophysica Acta

Changes in plasma membrane fluidity of *Bryonia dioica* internodes during thigmomorphogenesis

Catherine Mathieu [a,*], Claude Motta [b], Marie-Andrée Hartmann [c], Catherine Thonat [a], Nicole Boyer [a]

[a] *U.A. Physiologie Intégrée de l'Arbre Fruitier, INRA-Université Blaise Pascal, 4 Rue Ledru, 63 000 Clermont-Ferrand, France*
[b] *Laboratoire de Biochimie, Hôtel-Dieu, Clermont-Ferrand, France*
[c] *Département d'Enzymologie Cellulaire et Moléculaire de l'Institut de Biologie Moléculaire des Plantes (UPR CNRS 406), Strasbourg, France*

Received 13 September 1994; accepted 8 December 1994

Abstract

Fluidity changes in plasma membrane (PM) lipid extracts or native membranes isolated from *Bryonia dioica* internodes after a mechanical stimulation were monitored by steady-state fluorescence polarization with 1,6-diphenyl-1,3,5-hexatriene as a probe. The signal was shown to rapidly induce an increase in the bulk lipid fluidity. This event was closely related to a relative enrichment in some phospholipid species (PC, PG and PS) as well as a significant increase in the unsaturation index of total fatty acyl chains. Free sterols and protein content did not appear to be involved into this process. After 48 h, lipids from rubbed internodes became less fluid than PM lipids from control internodes.

Keywords: Plasma membrane fluidity; Steady-state fluorescence polarization; Sterol; Phospholipid; Fatty acid; (*Bryonia dioica*)

1. Introduction

Thigmomorphogenesis is an ubiquitous response of plants to mechanical perturbations (rubbing, flexing, shaking, wind) [1]. Mechanical stimulus applied to young internodes of *Bryonia dioica* results in reduced elongation and increased expansion [2]. Various biochemical events such as lignification [3,4], changes in endogenous auxin levels [5] or ethylene production [6–8] are known to occur during this process. A rapid leakage of Ca^{2+} from the plasma membrane (PM) surface, followed by an influx of these ions into the cytoplasm [9], the binding of some peroxidases to membranes [10] and changes in PM H^+-ATPase activity [11] are among early events of thigmomorphogenesis, which emphasize the involvement of membranes, in particular, the PM. The present paper is devoted to changes in the PM fluidity in response to a mechanical stimulus monitored by steady-state fluorescence polarization measurements with DPH as a probe. Results are discussed in relation to modifications of some PM components such as phospholipids, fatty acids, sterols and proteins during this process.

2. Material and methods

2.1. Plant material and growth conditions

Bryonia dioica plants were grown in 50 l containers in a growth room at 25 ± 1° C with a 16 h-photoperiod cycles of photosynthetically active white light (60 μmol/m^2 per s) and 60 ± 10% of relative humidity. The rubbing stimulus consisted in holding the last growing internode (±15 mm) between thumb and forefinger while gently rubbing up and down; anatomical data of these internodes [2] revealed hypertrophy and weak thickening of the cell wall of the sub-epidermal layer's cells. Control and rubbed internodes were excised at different times ranging from 20 min to 48 h after the treatment and used for PM isolation.

Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; ΔE, free energy of activation; FA, fatty acid; PM, plasma membrane; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; Sulfo PL, sulfophospholipid.

* Corresponding author. Fax: +33 73 346796.

SSDI 0005-2736(94)00313-0

2.2. Preparation of a plasma membrane

Young internodes were homogenized with a Waring C blendor (at a ratio of 4 ml per g of fresh weight) in 50 mM Hepes (pH 7.5) containing 400 mM sucrose, 100 mM KCl, 1 mM $MgCl_2$, 10 mM ascorbic acid, at 4° C. After filtration through nylon cloth (10 μm mesh), the homogenate was centrifuged at $10\,000 \times g$ for 10 min. The pellet was discarded and the supernatant was centrifuged at $40\,000 \times g$ for 30 min. The resulting supernatant was further centrifuged at $100\,000 \times g$ for 60 min to yield a pellet of microsomal membranes. This pellet was suspended in 1 ml of 5 mM potassium phosphate buffer (pH 7.8). Plasma membrane-enriched fractions were isolated using the aqueous two-phase polymer partitioning method of Kjellbom and Larson [12]. The resulting pellet (PM vesicle fraction) was resuspended in 5 mM potassium phosphate buffer (pH 7.8) to give a protein concentration of about 1 mg/ml. Preparations were stored at −80° C until used.

Protein was estimated by the method of Bradford [13] using bovine serum albumin as standard.

2.3. Lipid extraction

PM preparations were evaporated to dryness and resuspended in chloroform-methanol (2:1, v/v). Then, proteins were eliminated by filtration through $Na_2\ SO_4$ (500 mg). Lipids were dissolved in 1 ml of chloroform-methanol (2:1, v/v) containing butylated hydroxytoluene (100 mg/l).

2.4. Fractionation of membrane lipids

Total lipids were fractionated by using a silica Sep-Pack cartridge fixed on a syringe. Neutral lipids, glycolipids and polar lipids were eluted by chloroform (15 ml), acetone (15 ml) and methanol (15 ml), respectively.

2.5. Polar lipid separation

Components of the polar lipid fraction were dissolved in chloroform-methanol (2:1, v/v) and separated by TLC on silicagel plates (Merck G60) using chloroform/methanol/acetic acid/water (85:15:10:3.5, v/v) as a solvent system. After 2 h, phospholipids were visualized using iodine vapour.

2.6. Estimation of phospholipids

After TLC, the phosphorus content of total lipids and individual phospholipids was determined according to Jouanel et al. [14]

2.7. Quantitative analysis of fatty acids

The total lipid fraction was evaporated to dryness and resuspended in 2 ml of 5% KOH-methanol. Free fatty acids obtained by saponification at 80° C for 5 min were methylated by BF_3-methanol for 5 min at 80° C. After extraction (two times with two volumes of pentane), they were analysed by HPLC using a Hypersil 5 ODS (300 × 4 mm) Biochrom column at 25° C with methanol/water (90:10, v/v) as the mobile phase (flow rate 1 ml/min). Detection was by refractometry. Heptadecanoic acid was used as an internal standard. By comparison of their retention times with those of standards, seven FAs have been identified: linolenic acid, 12.6 min, palmitoleic acid, 13.7 min, myristic acid, 14.7 min, linoleic acid, 15.4 min, palmitic acid, 19.1 min, oleic acid, 20.1 min, stearic acid, 32.4 min.

The unsaturation index can be estimated using the following equation:

$$\frac{\text{mol\%(monounsaturated FA)} + 2\text{mol\%(diunsaturated FA)} + 3\text{mol\%(triunsaturated FA)}}{100}$$

2.8. Sterol analysis

Free sterols were analyzed and quantified according to Hartmann and Benveniste [15]. PM preparations were extracted three times with three volumes of hexane. Lipid extracts were subjected to TLC with dichloromethane as the developing solvent (2 runs). Only bands corresponding to 4-desmethylsterols were scraped off the silica. Sterols were analyzed as acetates by GC on a WCOT glass capillary column (25 m × 0.25 mm) coated with a DB1-phase using cholesterol as internal standard. The temperature program used included a fast rise from 60 to 230° C at 30° C/min, a slow rise from 230 to 280° C at 2° C/min and finally a plateau at 280° C for 5 min. Individual sterol components were identified by GC-MS of the acetate derivatives. *Bryonia dioica* internodes were found to contain a complex sterol mixture. By comparison of their RRTs and mass spectra with literature data [16,17], seven sterols have been identified: 24-methyl-5α-cholest-7-en-3β-ol, RRT 1.34, MS m/z (rel. int.): 442 (M^+, 100), 427 (18), 382 (6), 367 (11), 315 (11), 288 (7), 273 (9), 255 (59), 229 (29), 213 (35); 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol, RRT 1.37, MS m/z (rel. int.): 454 (M^+, 31), 439 (8), 411 (14), 379 (3), 351 (9), 342 (19), 315 (24), 313 (100), 288 (19), 273 (9), 255 (58), 229 (25), 213 (22); 24-ethyl-5α-cholesta-7,*E*-22,25(27)-trien-3β-ol, RRT 1.37, MS m/z (rel. int.): 452 (M^+, 12), 437 (3), 423 (5), 392 (2), 363 (4), 342 (19), 313 (100), 299 (8), 288 (19), 255 (58), 229 (25), 227 (8), 213 (22); 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol, RRT 1.42, MS m/z (rel. int.): 454 (M^+, 19), 439 (15), 394 (4), 379 (4), 342 (17), 315 (7), 313 (100), 299 (4), 288 (6), 273 (6), 255 (20), 229 (7), 228 (6), 227 (9), 213 (15); 24-ethyl-5α-cholest-7-en-3β-ol, RRT 1.45, MS m/z (rel. int.): 456 (M^+, 100), 441 (21), 396 (9), 381 (9), 315 (18), 288 (22), 273 (21), 255 (96), 229 (35), 213 (58); stigmasta-7,*E*-24(24^1)-dien-3β-ol (isoavenasterol), RRT 1.46, MS m/z (rel. int.): 454 (M^+,3), 439 (3), 379 (3), 356 (51), 313 (100), 296 (6), 255 (7), 253

(9), 229 (5), 227 (11), 213 (14); stigmasta-7,*Z*-24(24^1)-dien-3β-ol (Δ^7-avenasterol), RRT 1.49, MS *m/z* (rel. int.): 454 (M^+, 9), 439 (6), 379 (7), 356 (38), 341 (6), 313 (100), 296 (6), 288 (7), 281 (8), 255 (19), 253 (7), 213 (20).

2.9. Fluorescence polarization measurements

Lipid extracts were evaporated to dryness and labelled with 2 mM of 1,6-diphenyl-1,3,5-hexatriene (DPH) solution in tetrahydrofuran to give a final total lipid to probe molar ratio of 100–1000:1. After evaporation of tetrahydrofuran, lipid extracts were resuspended in 1 ml of 0.1 M phosphate buffer (PBS) (pH 7.4). The final suspension was then sonicated three times for 1 min every min and centrifuged at $10000 \times g$ for 15 min. 2 ml of PBS were added to the supernatant just before fluorescence polarization measurements.

PM fractions were resuspended in 3 ml of PBS and labelled with 2 mM DPH solution in tetrahydrofuran to give a final total lipid probe molar ratio of 100–1000:1. Suspensions were then incubated for 60 min in the dark at 37° C just prior to fluorescence measurements.

Fluorescence polarization measurements were made in an Aminco SPF 500 spectrofluorimeter equipped with a polarization fluorescence accessory. Measurements were performed between 15 and 45° C. The excitation and emission wavelengths were 360 and 460 nm, respectively. The samples were illuminated less than 10 s to avoid photoisomerisation of DPH. The fluorescence anisotropy r is defined as $r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ refer to the fluorescence intensities recorded through a polarization filter oriented alternatively in parallel and perpendicular to the vertically polarized excitation beam [18]. By application of Perrin's formalism for rotational depolarization of a fluorophore, the fluorescence anisotropy r of DPH in membranes has been correlated with the apparent 'microviscosity' η [18]. More precisely, the fluorescence anisotropy parameter $((r_0/r) - 1)^{-1}$, where r_0 is the limit anisotropy value in a rigid medium ($r_0 = 0.362$), is proportional to η and for comparative purposes can be used as a useful relative scale [18]. This microviscosity (or inversely the fluidity) is an average term which describes the bulk properties of the lipid bilayer. Data were expressed as Arrhenius plots of the anisotropy parameter over the range of 15 to 45° C.

Apparent energies of activation ΔE were calculated from the slopes of microviscosity Arrhenius plots below the break point [19]. This parameter showed the ability of the system to modify its fluidity according to a physical constraint (here, the temperature) [20].

2.10. Replications

Each figure represents the mean of three independent experiments. In all case a great reproducibility of the measurements was observed.

3. Results

3.1. Change in fluidity of PM lipid extracts and native PM preparations from Bryonia dioica internodes in response to the mechanical stimulus

Fluidity changes in PM lipid extracts from internodes isolated at different times after the stimulation were monitored by steady-state fluorescence anisotropy with DPH as a probe for temperatures ranging from 15 to 45° C and expressed as Arrhenius plots of the anisotropy parameter (Fig. 1). In all the cases, the anisotropy parameter of lipid extracts was found to decrease inversely to the rise of the temperature, indicating that, as expected, an increase in the mean fluidity was correlated with an increase in the temperature. Data fit best two-slope plots, with a breakpoint at 35 ± 2° C for all the preparations. Evidence is presented in Fig. 1 that the mechanical stimulation triggered a significant increase in fluidity of PM lipids. Such an effect could be detected as early as 20 min (Fig. 1a). Its amplitude, which increased after 4 h (Fig. 1b), was less important after 25 h (Fig. 1c). After 30 h, both PM lipid extracts exhibited approximately the same behavior (Fig. 1d). After 48 h, PM lipids from rubbed internodes even became less fluid than PM lipids from control internodes (Fig. 1e). The evolution in function of time after the signal of the difference in anisotropy parameter values recorded at 25° C between preparations from control and rubbed internodes is given in Fig. 2. The corresponding apparent energies of activation, calculated from the slopes of microviscosity Arrhenius plots between 15 and 35° C for the various preparations, are given in Table 1. After the signal, ΔE was decreased but after 36 h an opposite effect was observed, characterized by a higher ΔE in rubbed internode preparations than in control internode preparations.

A comparison of changes in fluidity between PM lipid extracts and native PM preparations from control and rubbed internodes isolated 4 h after stimulation is shown in Fig. 3. The DPH anisotropy parameter appears to be greater in the native membranes as compared to the lipid extracts, indicating that motional freedom of the probe is more restricted in the intact PM.

3.2. Fatty acid analysis

The total fatty acyl composition of PM preparations from control and rubbed internodes isolated 4 h after the stimulation is given in Fig. 4. The main FA of PM from control internodes was found to be linoleic acid (18:2), which accounts for 55% of total FAs. Myristic (14:0), stearic (18:0) and oleic (18:1) acids are present in much lower amounts. Linolenic acid (18:3) is just detectable. The mechanical stimulation triggered an increase in the proportions of oleic, linoleic and linolenic acids as well as a decrease in those of myristic and stearic acids with, as the consequence, a significant increase in FA unsaturation

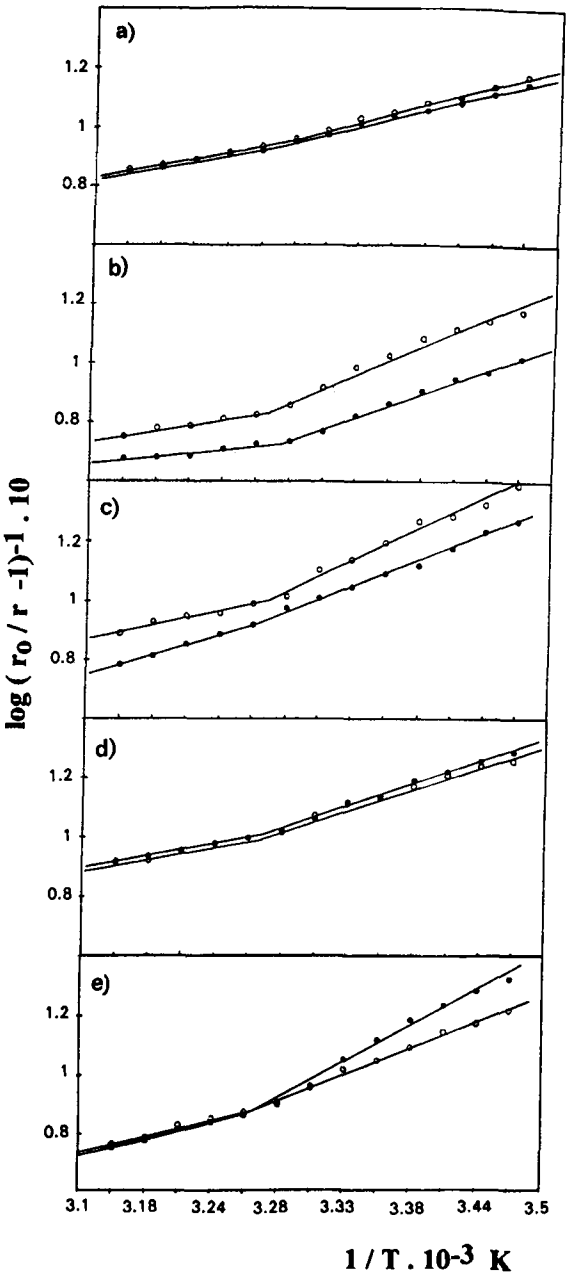

Fig. 1. Arrhenius plots of the fluorescence anisotropy parameter of PM lipidic extracts from control (○) and rubbed (●) internodes obtained 20 min (a), 4 h (b), 25 h (c), 30 h (d) and 48 h (e) after the stimulation.

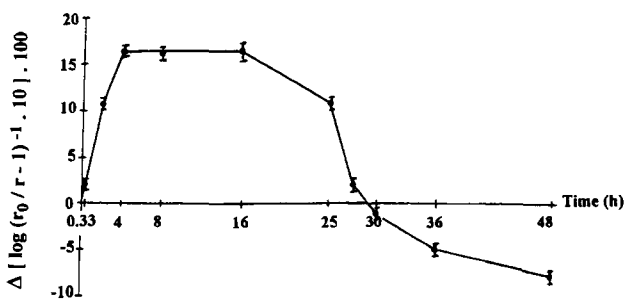

Fig. 2. Fluidity difference between control (○) and rubbed (●) internode PM lipidic extracts, presented as $\Delta\log(r_0/r-1)^{-1}$ at different times after rubbing, at 25° C.

Table 1
Apparent activation energy of PM lipidic extracts calculated from microviscosity Arrhenius plots below the break point

PM lipidic extracts	ΔE (kcal/mol)
Control	9.6
20 min	8.9
2 h	7.0
4 h	5.8
16 h	6.6
27 h 30 min	8.4
30 h	8.7
36 h	13.0
48 h	15.6

index of PM lipids from rubbed internodes (1.69 compared to 1.38 for control internodes).

3.3. Phospholipid analysis

The phosphatidylethanolamine (PE) was the most abundant phospholipid in PM from control internodes (Fig. 5). After the signal, the amount of this phospholipid as well as sulfophospholipid and phosphatidic acid (PA) amounts decreased. At the same time, the mechanical stimulus induced an important increase in phosphatidylinositol (PI) + phosphatidylserine (PS) (PI and PS were not dissociable) and phosphatidylcholine (PC) amounts. The phosphatidylglycerol (PG) amount did not fluctuate significantly.

3.4. Free sterol determination

Free sterol analysis was performed on control and rubbed internode PM preparations isolated at different times ranging from 0 to 30 h after the mechanical stimulation. All the preparations were found to contain a complex mixture of sterols. Chromatograms exhibited at least 8 peaks. Based on the GC and MS data, a total of seven 4-desmethylsterols were identified in the sterol mixture.

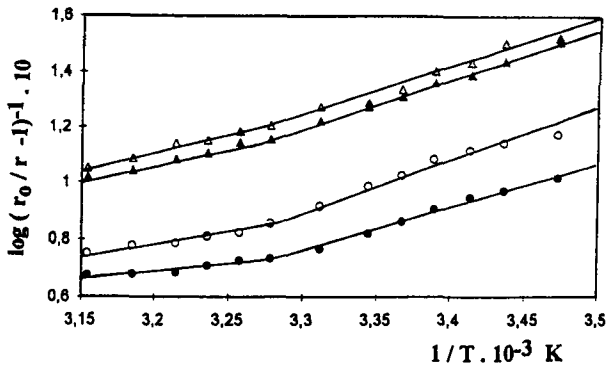

Fig. 3. Arrhenius plots of the fluorescence anisotropy parameter of native PM preparations (control (△) and rubbed (▲) internodes) and PM lipidic extracts (control (○) and rubbed (●) internodes). Rubbed internodes were obtained 4 h after the mechanical stimulus.

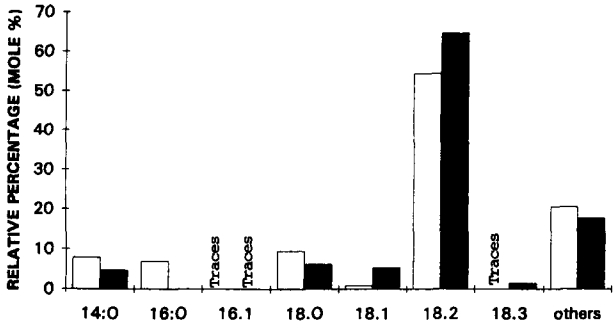

Fig. 4. Relative abundance (mol%) of fatty acids in PM isolated from control (□) and rubbed (■) internodes obtained 4 h after the mechanical stimulus. Myristic acid, (14:0); palmitic acid, (16:0); palmitoleic acid, (16:1); stearic acid, (18:0); oleic acid (18:1); linoleic acid, (18;2); linolenic acid, (18:3).

The peak 1 (RRT 1.32) contained two unidentified sterols having M^+ at m/z 440 and 454, respectively; the peak 2 (RRT 1.34) was identified to 24-methyl-5α-cholest-7-en-3β-ol; the peak 3 (RRT 1.37) consisted of a mixture of 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol (α-spinasterol), 24-ethyl-5α-cholesta-7,*E*-22,25(27)-trien-3β-ol and an unidentified sterol with M^+ at 456; the peak 4 (RRT 1.41) was a mixture of two unidentified sterols with M^+ at 454 and 456, respectively; the peaks 5 (RRT 1.42), 6 (RRT 1.45), 7 (RRT 1.46) and 8 (RRT 1.49) were identified to 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol, 24-ethyl-5α-cholest-7-en-3β-ol, stigmasta-7,*E*-24(24^1)-dien-3β-ol (isoavenasterol) and stigmasta-7,*Z*-24(24^1)-dien-3β-ol (Δ^7-avenasterol), respectively. This sterol composition is more or less similar to that reported earlier [21] in different parts of *Bryonia dioica* seedlings. In our case, however, 24-ethyl-5α-cholest-7-en-3β-ol was the most abundant sterol. *Bryonia dioica*, like other Cucurbitaceae, contains Δ^7-sterols whereas Δ^5-sterols, which are more common in higher plants, were not detected. The configuration of the C-24 alkyl substituent was not determined. Whereas Cucurbitacea sterols with the $\Delta^{25(27)}$ bond have been shown to have the β-orientation, the orientation at C-24 of sterols

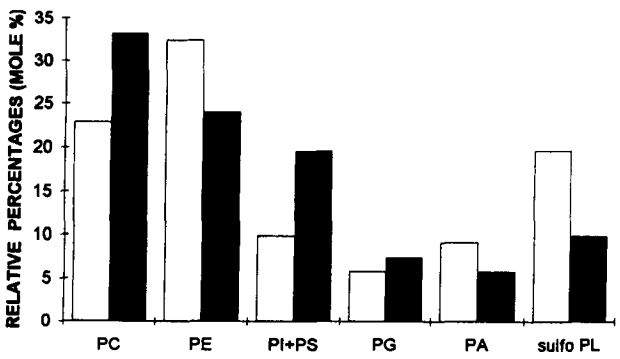

Fig. 5. Relative abundance (mol%) of phospholipids in PM isolated from control (□) and rubbed (■) internodes prepared 4 h after the mechanical stimulation. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid; SulfoPL, sulfophospholipid.

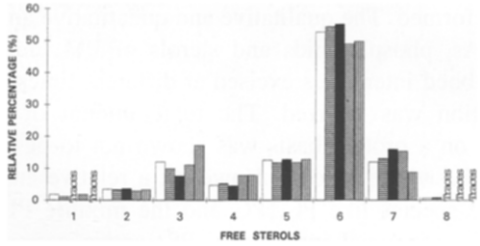

Fig. 6. Relative abundance of free sterols in the PM fractions from control (□), 30 h control (\\\) and rubbed internodes isolated 30 min (≡), 4 h (■), 30 h (|||) after the mechanical stimulation. Peak 2: 24-methyl-5α-cholest-7-en-3β-ol; peak 3: mixture of 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol and 24-ethyl-5α-cholesta-7,*E*-22,25(27)-trien-3β-ol; peak 5: 24-ethyl-5α-cholesta-7,*E*-22-dien-3β-ol; peak 6: 24-ethyl-5α-cholest-7-en-3β-ol; peak 7: stigmasta-7,*E*-24(24^1)-dien-3β-ol; peak 8: stigmasta-7,*Z*-24(24^1)-dien-3β-ol; peaks 1 and 4 contained a mixture of two unidentified free sterols.

lacking this double bound has been reported to change during development [22]. The absolute amounts in free sterols, expressed in μg/mg of protein, and the relative composition of each of these sterols in the different PM preparations are given in Fig. 6. No modification in both the qualitative and quantitative sterol composition of the PM preparations was observed in response to the mechanical stimulus. This was attested by constant sterol to phospholipid and sterol to protein ratios (Table 2).

4. Discussion

In the present paper, evidence is presented for a significant increase in the mean fluidity of PM lipids or native PM preparations from *Bryonia dioica* internodes in response to a mechanical stimulation. Such an effect could be detected as early as 20 min after the signal and was shown to reach its maximum magnitude after 4 h. After 30 h, control and rubbed internodes no longer exhibited a distinct behavior, and after 48 h, an opposite effect was even observed, characterized by a lower fluidity of PM lipids from rubbed internodes as compared to control internodes. In order to investigate whether these changes in PM fluidity could be correlated with modifications in the structural organization of the PM during thigmomorphogenesis, an extensive analysis of some PM components

Table 2
Total free sterol amounts of various PM preparations isolated from control and rubbed *Bryonia dioica* internodes

Extracts	Free sterols/ PLs molar ratio	Free sterols (μg/mg protein)
Control	0.50	56.3
Rubbed 30 min.	0.56	57.5
Rubbed 4 h	0.54	53.2
Control 30 h	0.49	55.3
Rubbed 30 h	0.48	54.4

was performed. The qualitative and quantitative analysis of total FAs, phospholipids and sterols of PM preparations from rubbed internodes excised at different times after the stimulation was realized. The total amount of PLs expressed on a protein basis was shown not to be modified during the whole process. However, a relative enrichment in some species like PC, PG and the mixture PI and PS (with a preferential increase in PS) and a corresponding decrease in the relative percentage of PE were observed. Such an effect is in favor of an increase in the PM bulk fluidity [20]. Concerning total FAs, the stimulation was shown to induce a significant increase in the unsaturation index, which can be held as responsible, at least in part, for the increase in the mean PM fluidity from rubbed internodes [23]. However, linolenic acid variations are involved in the tendril coiling response of *Bryonia dioica* [24]. As a matter of fact, touch rapidly initiates in tendrils the formation of jasmonate from α-linolenic acid, which is released as a messenger triggering the coiling response. So, a possible part of jasmonic acid in thigmomorphogenesis could be considered. Among components affecting the physical properties of the lipid bilayer, sterols are supposed to play an important role. In animals cells, the participation of cholesterol in regulating the PM fluidity has been well established. In particular, the mobility of the PL acyl chains, which is directly related to fluidity, is markedly decreased with cholesterol enrichment [20]. In contrast to most higher plants, in which sterols are present as Δ^5-sterols, with sitosterol, stigmasterol and 24-methylcholesterol as major components, *Bryona dioica* was found to contain a complex mixture of Δ^7-sterols as in other *Cucurbitaceae* [25]. Seven individual sterols were identified in the PM from *Bryonia* internodes. These sterols are similar to those already detected in the whole plant [21]. Previous work performed with model systems constituted of soybean PC bilayer has shown that sitosterol is very efficient in regulating the membrane fluidity and permeability and has been proposed to play in plant membranes a role similar to that of cholesterol in animal membranes [26,27]. In contrast, stigmasterol, a sterol which differs from sitosterol by an additional double bond at C-22, was found to be inefficient in ordering soybean PC bilayers. No study concerning effects of Δ^7-sterols on membrane fluidity has been carried out. When incorporated into egg PC liposomes, Δ^7-cholesterol was found to be far less effective than cholesterol in increasing the membrane order parameter [28]. In *Bryonia dioica* internodes, 24-ethylcholest-7-en-3β-ol is the major sterol and probably plays a role similar to that of sitosterol in this plant. Some sterols have an additional $\Delta^{25(27)}$ bond, which is supposed to affect the mobility of the sterol side chain and therefore the interactions with the acyl chains. As in other plants, PM preparations were shown to contain higher amounts of free sterols than intracellular membranes (data not presented). Both the qualitative and quantitative sterol composition of the PM from *Bryonia dioica* internodes was found not to be modified in response to the mechanical stimulation, from 30 min to 30 h (Fig. 6). Thus, sterols appear not to play a significant role in the change in PM fluidity induced by the thigmomorphogenesis process.

A comparative study between lipidic extracts and original PM preparations have shown that nature PM preparations were less fluid than lipidic extracts. That means a potential protein rigidifying effect. However, as the proteins/phospholipids ratio was not significantly modified [5,4] to [6,2], proteins would not play a major role in fluidity variations

In conclusion, in plasmalemma *Bryonia dioica* internodes, fluidity variations seemed principally regulated by fatty acids unsaturation and the polar heads of phospholipids.

Moreover, the stimulus effect on fluidity could be partly modulated by glycoproteins, glycolipids [29,30] and the cytoskeleton [31].

The Ca^{2+} rapid and transient release from the PM 30 s after the stimulation [9] constitutes a first wave of Ca^{2+} acting as a second messenger. This first step could participate to the perturbation of PM fluidity. In normal conditions Ca^{2+} has a stabilizing and tightening effect on the outer surface of the PM, ensuring maintenance of selective ionic permeability including that for itself. This effect of Ca^{2+} is explained by electrostatic interactions and involves bonding between phospholipids and phospholipids-proteins binding [32–34]. As a result of Ca^{2+} release the PM would become more fluid and then allow the opening of Ca^{2+} channels [20]. So, the primary consequence of mechanical stress on plant cells is the perturbation of cellular ion balance including pH resulting from increased passive fluxes and from cessation of PM H^+-ATPase activity during 10 min [11]. On the other hand, together with PM fluidity increase, the recovery and the stimulation of different ATP-dependent ion (H^+, Ca^{2+}) transport activities [11], would be involved in the restoration of a new ionic equilibrium. Relationships between these changes in membrane fluidity and PM functioning have to be investigated now. Changes in the lipid environment of the PM may regulate the activity of the ATPase [35]. PM ATPase purified from higher plants appears to require specific lipids. PC, PS and PG are effective stimulators of the activity of solubilized PM ATPase [35,36]. Moreover, activity of the solubilized PM ATPase might be regulated by the length and degree of saturation of the FA chains in phospholipids [36]. In PM *Bryonia dioica* internodes the enrichment in PC, PS and PG and the increase in the unsaturation index of FA chains after the stimulation could be correlated with the H^+-ATPase activity which is significantly increased 10 min after the signal and during the following two hours [11].

In the nature, membrane systems have developed many processes to maintain an optimal fluidity [20]. Nonphysiologic or pathologic states are always correlated with important fluidity variations [19]. In this regard, *Bryonia dioica*

physiological response to the stimulation was consistent with no change in the transition of temperature. In fact, *Bryonia dioica* has developed an important aptitude to increase the PM fluidity after a stimulation. This capacity is decreased by the stress, as shown by the free energy of activation decrease. Moreover, in our experimental model, 36 h after the mechanical stimulus, the increase in the anisotropy factor could reflect an accelerated senescence in rubbed plants.

We can postulate that physiological responses of plants to stress are under dependence of the capacity to respond (to aggression) by membrane fluidity changes. Fluidity variations 36 h after the stimulation could be correlated with fluidity variations in relation with senescence [37,38]. These phenomenons spread a long time. In contrast, the fluidity increase of *Bryonia dioica* PM happens rapidly after the mechanical stimulus and only spreads over some hours. So we can postulate that fluidity variations in response to stress can be correlated with signal transduction.

References

[1] Jaffe, M.J. and Forbes, S. (1993) Plant Growth Regul. 12, 313–324.
[2] Boyer, N., Gaspar, T. and Lamond, M. (1979) Z. Pflanzenphysiol. 93, 459–470.
[3] De Jaegher, G., Boyer, N. and Gaspar T. (1985) Plant Growth Regul. 3, 133–148.
[4] De Jaegher, G. and Boyer, N. (1987) Plant Physiol. 84, 10–11.
[5] Hofinger, H., Chapelle, B. and Gaspar T. (1979) Plant Physiol. S 63, 52.
[6] Boyer, N., Desbiez, M.O., Hofinger, M. and Gaspar, T. (1983) Plant Physiol. 72, 522–525.
[7] De Jaegher, G., Boyer, N., Bon, M.C. and Gaspar, T. (1987) Biochem. Physiol. Planzen 182, 46–56.
[8] Bourgeade, P., Espagnol, M.C., De Jaegher, G. and Boyer, N. (1990) Plant Physiol. Biochem. 28, 385–392.
[9] Thonat, C., Boyer, N., Penel, C., Courduroux, J.C. and Gaspar, T. (1993) Protoplasma 176, 133–137.
[10] Boyer, N. and Gaspar, T. (1980) C.R. Acad. Sci. Paris 291, D série, 577.
[11] Bourgeade, P. and Boyer, N. (1994) Plant Physiol. Biochem. 32, 661–668.
[12] Kjellbom, P. and Larsson, C. (1984) Physiol. Plant 62, 501–509.
[13] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[14] Jouanel, P., Motta, C., Delattre, J. and Dastugue, B. (1980) Clin. Chim. Acta 105, 173–181.
[15] Hartmann, M.A. and Benveniste, P. (1987) Methods Enzymol. 148, 632–650.
[16] Itoh, T., Kikuchi, Y., Tamura, T. and Matsumoto, T. (1981) Phytochemistry 20, 761–764.
[17] Cattel, L., Balliano, G. and Caputo, O. (1979) Phytochemistry 18, 861–862.
[18] Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394.
[19] Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133–149.
[20] Shinitzky, M. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. 1, pp. 1–51, CRC Press, Boca Raton.
[21] Cattel, L., Balliano, G, Caputo, O. and Delprino, L. (1980) Phytochemistry 19, 465–466.
[22] Fenner G.P. and Patterson G.W. (1992) Phytochemistry 31, 73–75.
[23] Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
[24] Weiler, E.W., Albrecht T., Groth B., Xia, Z.O, Luxem, M., Lib, H., Andert L. and Spengler, P. (1993) Phytochemistry 32, 591–600.
[25] Nes W.R., Krevitz, K., Joseph, J., Nes, W.D., Harris, B., Gibbons, G.F. and Patterson, G.W. (1977) Lipids 12, 511–527.
[26] Schuler, I., Duportail, G., Glasser, N., Benveniste, P. and Hartmann, M.A. (1990) Biochim. Biophys. Acta 1028, 82–88.
[27] Krajewski-Bertran, M.A., Milon, A. and Hartmann, M.A. (1992) Chem. Phys. Lipids 63, 235–241.
[28] Ranadive, G.N. and Lala, A.K. (1987) Biochemistry 26, 2426–2431.
[29] Uemura, M. and Steponkus, P.L. (1994) Plant Physiol. 104, 479–496.
[30] Palta, J.P., Whitaker, B.D. and Weiss L.S. (1993) Plant Physiol. 103, 793–803.
[31] Tsuji, A. and Ohnishi, S.I. (1986) Biochemistry 25, 6133–6139.
[32] Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152.
[33] Ohnishi, S. and Itoh, T. (1974) Biochemistry 13, 881.
[34] Gitler, C. and Montal, M. (1972) FEBS Lett. 28, 329.
[35] Kasamo, K. and Nouchi, I. (1987) Plant Physiol. 83, 323–328.
[36] Kasamo, K. (1990) Plant Physiol. 93, 1049–1052.
[37] Borochov, A., Halevy, A.H. and Shinitzky, M. (1982) Plant Physiol. 69, 296–299.
[38] Leshem, Y.Y. (1991) in Plant Signalling, Plasma Membrane and Change of State (Penel, C. and Greppin, H., eds), pp. 31–58, Laboratory of Plant Physiology, University of Geneva, Geneva.