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Elicitins trap and transfer sterols from micelles, liposomes and plant plasma membranes

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Abstract

Using elicitins, proteins secreted by some phytopathogenic Oomycetes (*Phytophthora*) known to be able to transfer sterols between phospholipid vesicles, the transfer of sterols between micelles, liposomes and biological membranes was studied. Firstly, a simple fluorometric method to screen the sterol-carrier capacity of proteins, avoiding the preparation of sterol-containing phospholipidic vesicles, is proposed. The transfer of sterols between DHE micelles (donor) and stigmasterol or cholesterol micelles (acceptor) was directly measured, as the increase in DHE fluorescence signal. The results obtained with this rapid and easy method lead to the same conclusions as those previously reported, using fluorescence polarization of a mixture of donor and acceptor phospholipid vesicles, prepared in the presence of different sterols. Therefore, the micelles method can be useful to screen proteins for their sterol carrier activity. Secondly, elicitins are shown to trap sterols from purified plant plasma membranes and to transfer sterols from micelles to these biological membranes. This property should contribute to understand the molecular mechanism involved in sterol uptake by *Phytophthora*. It opens new perspectives concerning the role of such proteins in plant–microorganism interactions.

Keywords: Cryptogein; Dehydroergosterol; Elicitin; Lipid–protein interaction; *Phytophthora*

1. Introduction

Phospholipids and sterols play major structural and functional roles in all the animal and plant cell membranes [1]. The enzymes necessary for these component biosyntheses are not present in all the endomembranes, but it was shown that lipid transfer proteins (LTP) ensure lipid transport between cell compartments [2,3]. For some LTP, the sterol binding properties and the sterol transfer activities have been largely studied [4–8].

Recently, preliminary results showed that small proteins excreted by *Phytophthora* spp., called elicitins [9], bind and transfer sterol between phospholipidic membranes [10,11]. Since the producing microorganisms are not able to synthesize sterols [12,13],

Abbreviations: BSA, bovine serum albumin; DHE, $\Delta^{5,7,9(11)22}$-ergostatetraen-3β-ol or dehydroergosterol; SCP, sterol carrier protein

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these proteins could be a tool for them to pick up these compounds from plant cell membranes. Thus, it would be interesting to determine how this sterol carrier activity is related to the largely studied hypersensitive response inducing activity of these proteins [14,15].

The lipid transfer activity of proteins can be detected either by radiochemical or by spectroscopic techniques. The radiochemical method involves the incubation of an acceptor membrane with ^{3}H-labeled donor membrane containing lipids. Thereafter, the membranes are separated and the radioactivity of acceptor membranes is analyzed. The fluorometric method consists in the fluorescence measurements of the mixture of donor vesicles (containing a self-quenched fluorescent lipid) and acceptor vesicles (containing a non-fluorescent lipid). When they are incubated together, a slight increase in fluorescence is observed resulting from the exchange of sterols between the two types of vesicles. The rate of fluorescence increase is markedly stimulated by the addition of a lipid transfer protein. This method is accurate and sensitive, and it has been used to study the transfer between membranes of different classes of lipids: fatty acids [16], phospholipids [17] and sterols [4,5,18–20].

In this paper, a simple fluorometric method to screen the sterol-carrier ability of proteins, avoiding the preparation of sterol-containing phospholipidic vesicles, is proposed. The transfer of sterols was measured directly between donor dehydroergosterol micelles and acceptor stigmasterol or cholesterol micelles, in the presence or absence of elicitins. The elicitin-induced increase of dehydroergosterol (DHE) fluorescence obtained with this method was compared to that determined with mixed sterol–phospholipid vesicles. This work establishes that elicitins are sterol-carrier proteins not only with artificial, but also with natural membranes, like purified tobacco plasma membranes.

2. Materials and methods

2.1. Chemicals, elicitors and plant materials

Sterols were purchased from Sigma and were dissolved in ethanol. The concentration of dehydroergosterol was corrected using molar extinction coefficient [21]. Elicitins (cactorein, capsicein, cryptogein, and parasiticein) were obtained as described earlier [9], dissolved in water and stored at −30°C.

2.2. Plasma membrane purification

Plasma membrane-enriched fractions were obtained following the aqueous partitioning procedure described by Widell et al. [22]. All steps of the isolation were performed at 4°C. After sowing, tobacco plants were grown in greenhouse for 45 days. Leaves (200 g) were homogenized in 400 ml of grinding medium (50 mM Tris-MES pH 8.0, 500 mM sucrose, 20 mM EDTA, 10 mM DTT and 1 mM PMSF) using a Waring blendor-homogenizer. The homogenate was filtered through a nylon screen (pore size, 75 μm) and then centrifuged at $16\,000\times g$ for 20 min. After centrifugation, the supernatants were collected, filtered through two successive screens (pore size, 63 and 38 μm, respectively) and centrifuged at $96\,000\times g$ for 35 min. The resulting supernatants were discarded and the pellets were suspended in PSK buffer (5 mM phosphate buffer pH 7.8, 300 mM sucrose, 3 mM KCl). Then, the microsomal fraction was added to an aqueous polymer two-phase system with final concentrations of 6.6% (w/w) dextran (500 000 MW), 6.6% (w/w) polyethylene glycol (3350 MW) in PSK buffer, then mixed by 40 tube inversions and centrifuged in a swing out rotor at $1200\times g$ for 5 min. The upper phase was carefully removed and washed two times with an equal volume of fresh lower phase. The last upper phase was diluted with 5 vols. of washing buffer (10 mM Tris-MES pH 7.3, 250 mM sucrose, 1 mM ATP, 1 mM EDTA, 1 mM DTT and 1 mM PMSF) and centrifuged at $96\,000\times g$ for 35 min. The pellet was suspended in the washing buffer and centrifuged at $120\,000\times g$ for 40 min. The resulting pellet was resuspended in 10 mM Tris-MES pH 6.5, 250 mM sucrose, 1 mM PMSF, 2 mM $MgCl_2$ and 20% glycerol, and stored at −80°C. Protein content of the plasma membrane preparations was measured according to Bradford's method [23], using BSA as standard and purity was determined using marker enzymes of other cell compartments as described by Larsson et al. [24]. Contamination did not exceed 5%.

2.3. Cryptogein treatments

Plasma membrane was diluted with buffer I containing 175 mM mannitol, 0.5 mM $CaCl_2$, 0.5 mM K_2SO_4, 2 mM MES, pH 5.75 (2 v/v). Aliquots containing 750 μg of plasma membrane proteins were incubated with 200 μg cryptogein, for 40 min at 15°C. Then, all the samples were centrifuged at $80\,000 \times g$ for 40 min, at 15°C. From the supernatants, aliquots were removed for protein quantitation, and sterol extractions were performed. The pellets were resuspended in 1 ml buffer I and membranar sterols were extracted. Controls were carried out with plasma membrane untreated with cryptogein.

2.4. Sterol extraction and quantitation

For each extraction, cholesteryl methyl ether was used as standard. Sterols from plasma membrane supernatants were extracted three times with chloroform (1/10 v). The organic extracts were concentrated to dryness with a rotatory evaporator.

Sterols from plasma membrane pellets were extracted according to Bligh and Dyer's method [25].

Sterol determination was performed using a Nermag R10-10 mass spectrometer. Quantitation was performed using a Perkin–Elmer Autosystem equipped with a Supelco Sac-5 column (30 m × 0.25 mm × 0.25 μm), a FID detector and a PE Nelson model 1020 computer. Gas flows were set at 1, 45 and 450 ml/min for nitrogen, hydrogen and air, respectively. The oven temperature rose from 50 to 285°C, 45°C/min and was kept at 285°C for 30 min. A calibration curve was obtained using cholesterol, campesterol, stigmasterol and β-sitosterol purchased from Supelco. Cholestane was used as calibration standard.

2.5. Fluorescence measurements

Fluorescence measurements were performed at 25°C with a Shimadzu RF 5001 PC spectrofluorometer in a stirred fluorometric cuvette with 2 ml of buffer II containing 175 mM mannitol, 0.5 mM $CaCl_2$, 0.5 mM K_2SO_4, and 5 mM MES, pH 7.0. For DHE, the excitation and emission wavelengths were set at 325 and 370 nm, respectively. The fluorescence of elicitins was negligible. Fluorescence intensity was expressed in arbitrary units (a.u.). Measurements of elicitin-induced sterol exchanges were performed using stigmasterol or cholesterol micelles (3 μM) added to 2 ml buffer III (10 mM MES pH 7.0 and 0.02% azide), containing DHE micelles (0.63 μM). Fluorescence of DHE was then recorded in absence (spontaneous transfer) or in presence of elicitins. In order to study the transfer of sterol from a sterol dry film to the solution, 2 μl of ethanolic DHE solution (2 nmol) was dried on the wall of the spectrofluorometer cuvette. Thereafter, 2 ml of buffer III were added and fluorescence of DHE was measured before, and after, the addition of cryptogein.

3. Results and discussion

3.1. Interaction of sterol micelles with elicitins

Elicitins are small (10-kDa) hydrophilic holoproteins, containing 98 amino acids, three disulfide bridges [9] and a hydrophobic core [26]. Their addition to DHE results in a marked fluorescence increase at 370 nm, the wavelength corresponding to the maximal emission of DHE, showing interaction between elicitins and DHE. Moreover, this increase was used to calculate the binding parameters of sterol to the elicitins. The number of binding sites was shown to be between 0.86 and 1.16 per elicitin molecule and the dissociation constants of the elicitin–sterol complexes are about 0.5 μM [11]. Fig. 1 shows binding plots of the interaction between sterols and cryptogein in mixed DHE–cholesterol or DHE–stigmasterol micelles. DHE and stigmasterol were mixed in ethanol before adding to the fluorometric cuvette. The concentration of DHE varied and that of the other sterol was constant. The curves confirm a competition between the fluorescent and the non-fluorescent sterol and show that the interaction between sterols and cryptogein was not sterol-selective.

3.2. Transfer of sterols by elicitins, using sterol micelles

In water, DHE forms micelles with a critical micellar concentration of 0.025 μM [5]. Fluorescence

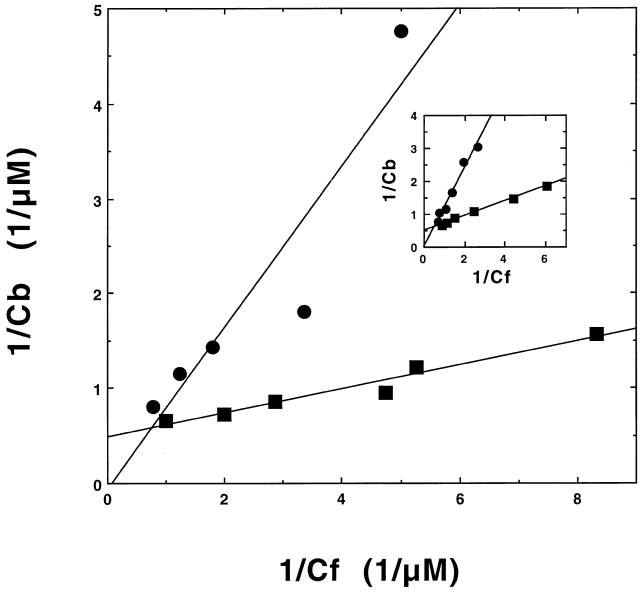

Fig. 1. Binding plots of the titration of cryptogein by DHE. Cb and Cf are the concentrations of bound and free DHE, respectively. Experiments were performed with 2.5 μM cryptogein, (■) in the presence of DHE alone, (●) in the presence of DHE+ cholesterol (0.83 μM, main curve) or DHE+stigmasterol (0.83 μM, inset). The results are mean values of two experiments. The values of the apparent dissociation constants (K_d) were 0.26 and 0.45 μM, and those of the numbers of sites (n) were 1.03 and 1.00 for the main curve and the inset, respectively.

quantum yield of DHE in micelles is low because the DHE molecules interact to self-quench [6]. At the starting point of the experiment, all DHE molecules were packed in donor micelles. Then, when micelles of a non-fluorescent sterol were added to DHE micelles, a slight fluorescence increase was observed ('spontaneous exchange' Fig. 2), due to a dilution and to a separation of DHE molecules. The fluorescence of DHE, in premixed DHE–stigmasterol micelles (at the same concentrations as previously), was 8-fold higher than that in pure DHE micelles. The addition of cryptogein to the mixture of donor and acceptor micelles stimulated the increase in fluorescence of DHE which reached a plateau after about 4 min (Fig. 2). This increase in fluorescence is due to the dilution of DHE in stigmasterol micelles and not to the fluorescence of a DHE–elicitin complex. The initial rate of the fluorescence increase was proportional to the cryptogein concentration, but the fluorescence after equilibration was the same (data not shown). The contribution of the complex DHE–elicitin in the fluorescence increase could be neglected because these experiments were performed with an excess of non-fluorescent sterol, which competes with DHE (Fig. 1). This result shows that cryptogein could stimulate the exchange of sterols between these two types of micelles. The other elicitins assayed were able to catalyze the same exchange of sterols between micelles but to a lower extent. Cactorein was somewhat less efficient than cryptogein, whereas the effects of parasiticein and capsicein were lesser (Fig. 2). On the other hand, the addition of BSA to the mixture of sterol micelles causes only a slight change in fluorescence of DHE [10]. BSA is able to bind non-specifically hydrophobic substances and has six to seven binding sites of sterol per molecule of protein with a lower affinity (K_d = 2.9 μM) [5] than elicitins. Evidently, BSA is not able to catalyze transfer of sterol between DHE and stigmasterol micelles (Fig. 2). Similar results were obtained using cholesterol instead of stigmasterol micelles (data not shown).

The fluorescence changes could result from a fusion of both types of micelles or a transfer of indi-

Table 1
Comparison of the kinetics of sterol transfer between donor–acceptor micelles and between donor–acceptor small unilamellar vesicles (SUV) (66% phosphatidylcholine, 12% phosphatidylserine and 22% sterols w/w)

Elicitin	Initial rate of fluorescence increase in micelles[a] (a.u. min^{-1})	Initial rate of fluorescence polarization increase in SUV[b] (min^{-1})
None	0.50	0.025
Capsicein	0.71	0.035
Parasiticein	1.33	0.050
Cactorein	1.94	0.070
Cryptogein	14.00	0.120

[a]Initial rates of fluorescence increase were calculated using the smoothed curves shown in Fig. 2.
[b]Initial rates of fluorescence polarization increase were calculated using data from Mikes et al. [11].

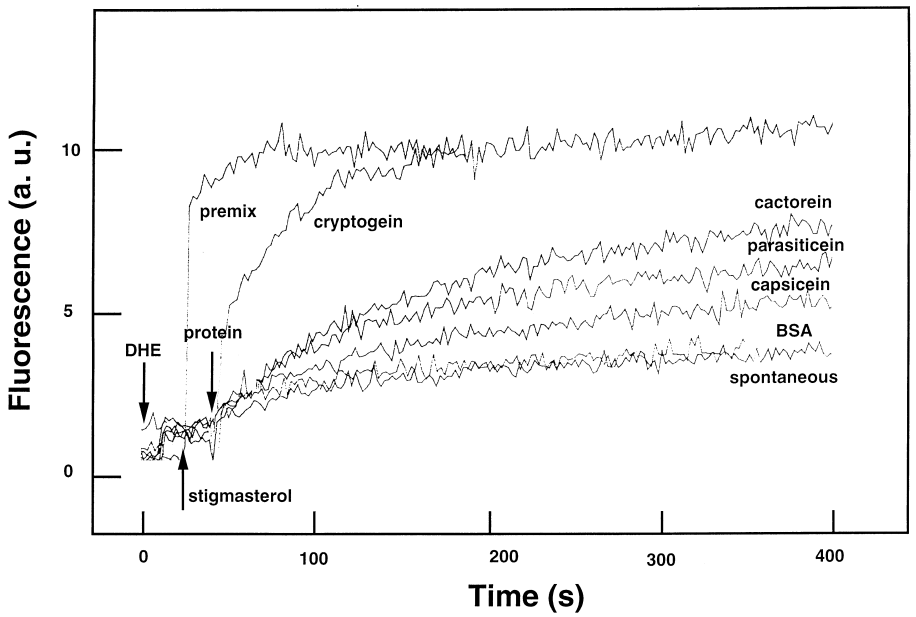

Fig. 2. Transfer of sterols between DHE and stigmasterol micelles catalyzed by elicitins followed by the changes of DHE fluorescence. Donor micelles contained DHE (0.63 μM), acceptor micelles stigmasterol (3 μM), in buffer III. The transfer was induced by adding elicitins or BSA (0.5 μM). In premix experiments, DHE and stigmasterol were mixed in ethanol before adding to the fluorometric cuvette.

vidual sterol molecules between these micelles. In order to impeach the fusion mechanism in the fluorescence increase, we dried DHE on the wall of the fluorometric cuvette. As shown in Fig. 3, no significant increase in fluorescence occurred after the addition of buffer into the DHE-coated cuvette. On the other hand, the addition of cryptogein to buffer, in the DHE-coated cuvette, resulted in an increase in fluorescence due to the formation of a cryptogein–DHE complex. The addition of acceptor cholesterol micelles together with cryptogein gave rise to a large increase in DHE fluorescence, whereas the addition of cholesterol micelles without cryptogein had no effect. The mechanism of this increase is evidently a cryptogein-induced transfer of individual DHE molecules from the cuvette wall to micelles of cholesterol. An assay with a double-coated cuvette (with DHE on one wall and cholesterol on the opposite one) allows to confirm the competition between DHE and other sterols. So, cryptogein is able to transfer individual sterol molecules from the cuvette wall to acceptor micelles and the same mechanism of the transfer is probable between donor DHE micelles and acceptor stigmasterol micelles.

3.3. Comparison between elicitin-induced sterol transfer in micelles, and in liposomes

The results obtained with the micelle method were compared with those reported using fluorescence polarization measurements of sterol transfer, catalyzed by these elicitins between phospholipid unilamellar vesicles. Table 1 shows the initial rates of fluorescence increase after the addition of elicitins to micelles calculated on the basis of the fluorescence measurements shown in Fig. 2. These values were compared with the initial rates of increase in fluorescence polarization in phospholipid vesicles published earlier [11]. Both methods gave comparable results. Cryptogein was the most efficient, whereas capsicein proved to be only slightly efficient.

3.4. Cryptogein loading and sterol transfer between purified tobacco plasma membranes and micelles

When cryptogein was incubated with tobacco plasma membrane, about 43% of the elicitin was retrieved in the supernatant after ultracentrifugation. Cryptogein interacting with plasma membranes was

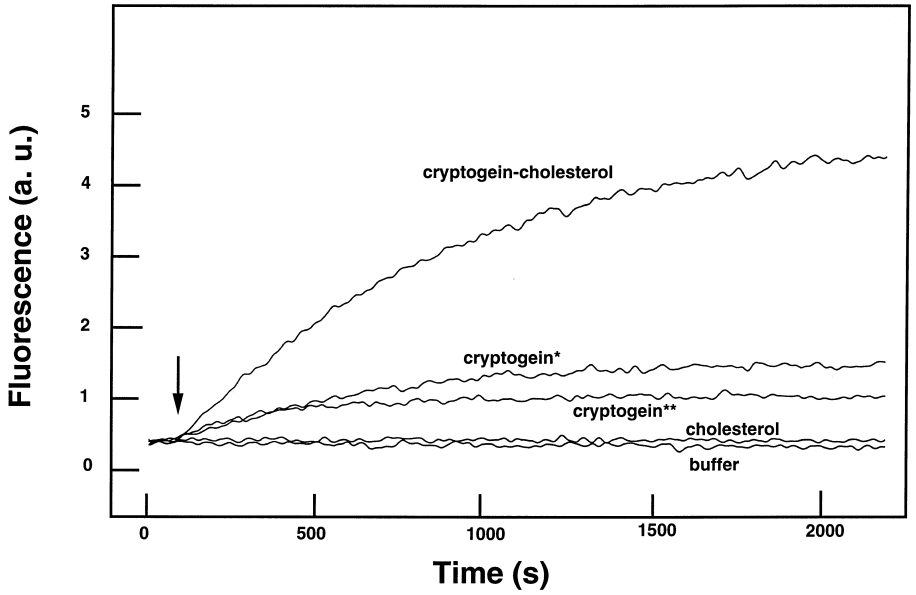

Fig. 3. Removal of DHE adsorbed on the wall of the fluorometric cuvette by cryptogein. DHE (2 nmol) was dried on the cuvette wall. Fluorescence was followed after the addition of 2 ml of buffer III containing cryptogein* (0.25 μM) and cholesterol micelles (5 μM) as indicated. Then, the experiment was repeated with 2 nmol cholesterol and 2 nmol DHE dried on the opposite sides of the cuvette with 2 ml buffer III containing cryptogein** (0.25 μM).

able to pick up sterols. Loading of elicitin was not selective (random uptake) and represented 6.6% of the total sterol contents of the plasma membranes (Table 2), but the molar ratio sterol/cryptogein found in the supernatant was 0.4–1.0. Moreover, cryptogein catalyzed a transfer of DHE from pure micelles to plasma membranes of tobacco. This led to an increase in fluorescence of DHE in the presence of plasma membrane and cryptogein (Fig. 4). Fluorescence of DHE–cryptogein complex was low. The total concentration of membrane sterols and DHE in this experiment was calculated. It was about 1.6 μM and 1 μM, respectively, and in these conditions all sterols must compete in binding to cryptogein. No effect was observed when cryptogein was replaced by BSA.

These observations suggest that cryptogein interacts with biological membranes, in the same way as that already reported with sterol micelles, or liposomal membranes containing sterols. They provide good evidence for a possible molecular activity of elicitins during plant–*Phytophthora* interactions. The fluorescence method is indirect, but it is rapid and simple without separation of donor and acceptor membranes. The exchange kinetics of DHE and [^{3}H]cholesterol did not differ significantly when present in the same donor membrane [27]. It confirms the reliability of the fluorometric method

Table 2
Sterol uptake by cryptogein from plasma membrane of tobacco leaves

	Amount of sterols bound to cryptogein (μg/mg PM protein)	Total amount of plasma membrane sterols (μg/mg PM protein)	% Uptake
Cholesterol	1.2 ± 0.6	17.0 ± 2.1	6.7
Campesterol	2.0 ± 0.7	33.3 ± 4.7	6.0
Stigmasterol	3.4 ± 1.0	60.4 ± 7.2	5.6
β-Sitosterol	1.9 ± 0.2	18.7 ± 3.5	9.9
Total	8.5 ± 2.1	129.4 ± 17.4	6.6

The results represent the mean ± S.D. of four repetitions, the amount of sterol extracted in control supernatants has been subtracted.

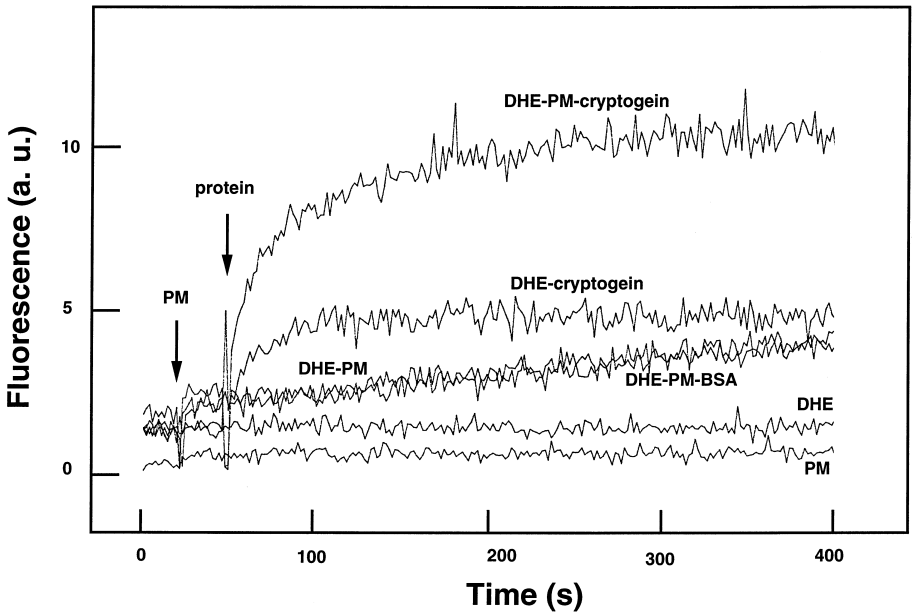

Fig. 4. Sterol transfer from DHE micelles to purified tobacco plasma membrane induced by cryptogein. Fluorescence of DHE in 2 ml of buffer III was followed in the presence of cryptogein, BSA and plasma membrane vesicles (PM) as indicated. Concentration of DHE was 1 μM, cryptogein and BSA 0.5 μM, plasma membrane 10 μg protein/assay.

[7,8]. The binding of sterol to cryptogein could be similar as that to non-specific sterol carrier protein (SCP2) which bind sterol and enhances sterol transfer between plasma membrane and mitochondrial or microsomal membranes. The separation of donor and acceptor membranes by dialysis membrane non-permeable to SCP2 did not inhibit the sterol transfer although the formation of the complex was necessary for the transfer [7]. So, it seems that SCP2 only stimulates the sterol desorption from the membrane, but this desorption is not a limiting step of the transfer [8].

4. Conclusion

We present a simple fluorometric method to test a sterol-carrier ability of proteins avoiding the preparation of mixed sterol-phospholipid vesicles. The transfer of sterols was measured directly between donor DHE micelles and acceptor stigmasterol or cholesterol micelles. Cryptogein traps sterols from purified plasma membrane of plants and catalyzed their transfer from sterol micelles to plasma membrane. The sterol species picked from these biological membranes seemed to be directly related to the sterol composition of the target membrane.

Our results afford the first evidence for a molecular activity of elicitins which appears to be extracellular sterol carrier proteins. This opens new perspectives concerning the role of such proteins in plant–microorganism interactions.

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References

[1] A.R. Slabas, T. Fawcett, Plant Mol. Biol. 19 (1992) 169–191.
[2] J.-C. Kader, Biochim. Biophys. Acta 380 (1975) 31–44.

[3] J.-C. Kader, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 627–654.
[4] R.T. Fischer, M.S. Cowlen, M.E. Dempsey, F. Schroeder, Biochemistry 24 (1985) 3322–3331.
[5] F. Schroeder, P. Butko, G. Nemecz, T.J. Scallen, J. Biol. Chem. 265 (1990) 151–157.
[6] F. Schroeder, P. Butko, I. Hapala, T.J. Scallen, Lipids 25 (1990) 669–674.
[7] J.K. Woodford, S.M. Colles, S. Myers-Payne, J.T. Billheimer, F. Schroeder, Chem. Phys. Lipids 76 (1995) 73–84.
[8] A. Frolov, J.K. Woodford, E.J. Murphy, J.T. Billheimer, F. Schroeder, J. Biol. Chem. 271 (1996) 16075–16083.
[9] P. Ricci, P. Bonnet, J.-C. Huet, M. Sallantin, F. Beauvais-Cante, M. Bruneteau, V. Billard, G. Michel, J.-C. Pernollet, Eur. J. Biochem. 183 (1989) 555–563.
[10] V. Mikes, M.-L. Milat, M. Ponchet, P. Ricci, J.-P. Blein, FEBS Lett. 416 (1997) 190–192.
[11] V. Mikes, M.-L. Milat, M. Ponchet, F. Panabières, P. Ricci, J.-P. Blein, Biochem. Biophys. Res. Commun. 245 (1998) 133–139.
[12] J.W. Hendrix, Annu. Rev. Phytopathol. 8 (1970) 111–130.
[13] J.D. Weete, Adv. Lipid Res. 23 (1989) 115–165.
[14] P. Ricci, F. Panabières, P. Bonnet, N. Maïa, M. Ponchet, J.-C. Devergne, A. Marais, L. Cardin, M.-L. Milat, J.-P. Blein, in: B. Fritig, M. Legrand (Eds.), Mechanisms of Plant Defense Responses, Kluwer Academic Publishers, Dordrecht, 1993, pp. 121–135.
[15] H. Keller, J.-P. Blein, P. Bonnet, E. Bourdon, F. Panabières, P. Ricci, in: M. Daniel (Ed.), Advances in Molecular Genetics of Plant–Microbe Interactions, Kluwer Academic, Dordrecht, 1994, pp. 327–332.
[16] S.R. Sha, C.D. Kane, Z. Xu, L.J. Banashak, D.A. Bernlohr, J. Biol. Chem. 268 (1993) 7885–7892.
[17] F. Moreau, J.D. Devirville, M. Hoffelt, F. Guerbette, J.C. Kader, Plant Cell Physiol. 35 (1994) 267–274.
[18] F. Schroeder, Y. Barenholz, E. Gratton, T.E. Thompson, Biochemistry 26 (1987) 2441–2448.
[19] G. Smutzer, Biochim. Biophys. Acta 958 (1988) 323–333.
[20] G. Smutzer, Biochim. Biophys. Acta 946 (1988) 270–280.
[21] G. Smutzer, B.F. Crawford, P.L. Yeagle, Biochim. Biophys. Acta 862 (1986) 361–371.
[22] S. Widell, T. Lundberg, C. Larsson, Plant Physiol. 70 (1982) 1429–1435.
[23] M.M. Bradford, Anal. Biochem. 72 (1976) 248–254.
[24] C. Larsson, M. Sommarin, S. Widell, in: H. Walter, G. Johansson (Eds.), Aqueous Two-Phase Systems, Vol. 228, Academic Press, San Diego, CA, 1994, pp. 451–469.
[25] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[26] G. Boissy, E. de La Fortelle, R. Kahn, J.C. Huet, G. Bricogne, J.C. Pernollet, S. Brunie, Structure 4 (1996) 1429–1439.
[27] G. Nemecz, R.N. Fontaine, F. Schroeder, Biochim. Biophys. Acta 943 (1988) 511–521.