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Elicitins, Proteinaceous Elicitors of Plant Defense, Are a New Class of Sterol Carrier Proteins[1]

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Some phytopathogenic fungi within *Phytophthora* species are unable to synthesize sterols and therefore must pick them up from the membranes of their host-plant, using an unknown mechanism. These pseudo-fungi secrete elicitins which are small hydrophilic cystein-rich proteins. The results show that elicitins studied interact with dehydroergosterol in the same way, but with some time-dependent differences. Elicitins have one binding site with a similar strong affinity for dehydroergosterol. Using a non-steroid hydrophobic fluorescent probe, we showed that phytosterols are able to similarly bind to elicitins. Moreover, elicitins catalyze sterol transfer between phospholipidic artificial membranes. Our results afford the first evidence for a molecular activity of elicitins which appears to be extracellular sterol carrier proteins. This property should contribute to an understanding of the molecular mechanism involved in sterol uptake by *Phytophthora*. It opens new perspectives concerning the role of such proteins in plant-microorganism interactions, since elicitins trigger defence reactions in plants.

Key Words: **dehydroergosterol; lipid-protein interaction; fluorescence polarization; fluorescence spectroscopy.**

In animal and plant cells, phospholipids and sterols are important components of membranes. Because the enzymes governing their biosynthesis are not associated with all the endomembranes, membrane biogenesis requires transfer of newly synthesized compounds [1]. Twenty years ago, lipid-transfer proteins (LTPs) have been discovered [2] [for a recent review see [3]]. More recently, non-specific lipid transfer proteins like rat liver sterol carrier protein-2 (SCP2) have been described. They constitute a class of intracellular, hydrophobic ligand binding/transfer proteins [4-8]. These last proteins bind cholesterol and some of them catalyze its intermembranar transfer [6].

Beside the crucial role played by sterols in membrane functions [9], sterols stimulate growth of many organisms, acting like hormones. Growth and reproduction of fungi depend on the sterol metabolism [10, 11]. In contrast to the other Oomycetes, species of *Phytophthora* and *Pythium* genera do not synthesize sterols [10, 11] and therefore must pick them up from their host-plant, using a mechanism which is still unknown. Nevertheless, these fungi excrete proteins in their environment [12], the role of which remaining undetermined. The proteins synthesized by *Phytophthora*, named elicitins, have been extensively studied in the last years. They are small (10 kDa) hydrophilic holoproteins containing 98 amino acids, having a hydrophobic pocket and three disulfide bridges [13, 14]. They did not exhibit any protease, β-glucanase or phospholipase activity [15], and no other enzymatic activity has been reported so far. The structural characteristics of elicitins described above are similar to those of lipid-transfer proteins [16] or of sterol carrier proteins-SCP2 [6], despite different primary structures. This led us to investigate their possible sterol transfer activity.

Preliminary results, using cryptogein which is the elicitin secreted by *P. cryptogea*, showed that it exhibits

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The abbreviations used are**:** BSA, bovine serum albumin; DHE, $\Delta^{5,7,9(11)22}$-ergostatetraen-3β-ol or dehydroergosterol; PC, phosphatidylcholine; PS, phosphatidylserine; SCP, sterol carrier protein; SUV, small unilamellar vesicles; TNS, 2-p-toluidinonaphtalene-6-sulfonate.

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a sterol carrier activity [17]. Then, we were interested to know if this was a general property of elicitins. In this paper, the binding of DHE to four elicitins (cactorein from *P. cactorum*, capsicein from *P. capsici*, parasiticein from *P. parasitica* and cryptogein) is described. This fluorescent sterol has been previously used to study the interactions between sterols and macromolecules or membranes [4-6, 18, 19]. Moreover, we analyzed the changes in TNS fluorescence, a non-steroid hydrophobic fluorescent probe, as the result of the binding of five sterols (campesterol, cholesterol, dehydroergosterol, β-sitosterol and stigmasterol) to cryptogein. Finally, we studied the ability of elicitins to catalyze a sterol transfer between phospholipidic artificial membranes.

MATERIALS AND METHODS

Chemicals. All phospholipids, TNS and sterols were purchased from Sigma (S^t Quentin Fallavier, France). Elicitins (cactorein, capsicein, cryptogein, and parasiticein) were obtained as described earlier [20], dissolved in water and stored at −30°C. Elicitin purity has been monitored as described previously [21]. Sterols and TNS were dissolved in ethanol. The concentration of dehydroergosterol was corrected using the molar extinction coefficient [18].

Fluorescence measurements. Fluorescence measurements were performed at 25°C with a Shimadzu RF 5001 PC spectrofluorimeter (Roucaire, Paris, France) in a stirred fluorometric cuvette with 2 ml of measuring medium (buffer I) containing 175 mM mannitol, 0.5 mM $CaCl_2$, 0.5 mM K_2SO_4, and 5 mM MES, pH 7.0. For DHE and TNS, the excitation wavelengths were set at 325 and 355 nm and emission wavelengths at 370 and 430 nm, respectively. The fluorescence of cryptogein alone was negligible. Fluorescence intensity was expressed in arbitrary units (a.u.).

Interaction of elicitin with dehydroergosterol. To 0.2-3.8 μM DHE in buffer I (fluorescence intensity Fo) elicitin was added (2.0 μM). Fluorescence intensity (F) of the mixture was read after stabilization. From these measurements, the fluorescence titration curve ΔF *vs.* DHE concentrations was drawn (ΔFfl-Fo). Titration of a constant DHE concentration (0.25 μM) with elicitins until a constant fluorescence intensity was reached allowed to determine the fraction of bound ligand. To obtain the dissociation constants and the number of non-interactive binding sites, plots of 1/bound DHE *vs.* 1/free DHE were made on the basis of the following equation: $1/C_b = (K_d/NA).1/C_f + 1/NA$, where C_b and C_f are the concentrations of bound and free DHE, respectively, K_d the dissociation constant, A the concentration of acceptor and N the number of binding sites.

Isolation of a cryptogein-stigmasterol complex. Cryptogein was dissolved in buffer I (970 μg/948 μl) and an ethanolic solution of stigmasterol was added slowly (52 μl of a 1 mg/ml solution). After a 20 min incubation period, the cryptogein-stigmasterol complex was isolated from the excess of sterol by gel filtration through a G-25 Sephadex (Pharmacia, Saclay, France) column (125 mg equilibrated with the same buffer). The elution of the protein with buffer I was monitored by UV at 280 nm and the excess of sterol adsorbed on Sephadex was eluted by adding ethanol to the buffer. The quantitation of sterol was performed by GC analysis, using a Perkin Elmer Autosystem (Lyon, France) equipped with a FID detector and a Supelco (S^t Quentin Fallavier, France) SAC-5 capillary column (30 m, 25 mm ID, 0.25 μm) in the following conditions: N_2 as gas carrier (20 cm/s), oven temperature varying from 50 °C to 285 °C (45 °C/min), injector and detector temperatures set at 300 ° C.

Preparation of liposomes. Two types of SUV were prepared: donor vesicles contained PC, PS and DHE, acceptor vesicles contained PC, PS and cholesterol. Phospholipids and sterols were dissolved in chloroform. For each assay, 4.4 mg PC, 0.8 mg PS and 1.5 mg sterol (cholesterol or DHE) were mixed, chloroform was evaporated under nitrogen and traces of solvent were vacuum-evaporated for at least 1 h. Thereafter, 1 ml of exchange buffer (buffer II) containing 10 mM MES (pH 7.0) and 0.02% azide was added. The mixture was vortexed under nitrogen and then sonicated for three 5-min periods at 40 °C. The suspension of liposomes was centrifuged (150 000 g, 1 h) and the supernatant containing SUV was used for measurements. The recovery in SUV estimated on the basis of DHE fluorescence before and after centrifugation was about 60%.

Measurements of cryptogein-mediated sterol exchange between liposomes. Measurements of the steady-state polarization of fluorescence in liposomes were performed at 25°C with a Kontron SFM-25 spectrofluorimeter (Lyon, France) equipped with polarizers. In each exchange assay, 280 μg of SUV were used in 2 ml of buffer II so that the donor:acceptor ratio was 1:9. The intensities of vertically and horizontally oriented components were read during 10 min. The values obtained without DHE were subtracted.

Comparaison of elicitin sequences with protein databases. Similarities with non redondant protein databases were searched using BLAST [22] and FASTA [23] programs.

RESULTS

Interaction of DHE with elicitins. The uncorrected emission maxima of DHE excited at 325 nm in buffer I were 370, 402 and 424 nm [17]. The addition of any elicitin to DHE in the same buffer resulted in a marked increase in the emission maximum at 370 nm. The shape of the emission fluorescence spectra of all elicitin-DHE complexes were similar (data not shown). These interactions were time-dependent (Fig. 1): the fluorescence kinetic curves of the protein-DHE interactions displayed some differences. In our experimental conditions, the interaction rate was high in the case of cryptogein and cactorein and a plateau was reached after 100 s and 200 s, respectively. Fluorescence gradually increased for capsicein and parasiticein (Fig. 1) and reached an equilibrium after 5 min. Molecular weights of these extracellular proteins are about 10 kDa, but their primary structures and pIs are different: cryptogein is basic whereas cactorein, parasiticein and capsicein are acidic [20]. We analyzed the effect of the pH on the interaction between DHE and two of them: a basic one, cryptogein and an acidic one, capsicein. The fluorescence kinetic curves of the interaction were determined in buffer I adjusted at different pH values between 4.5 and 8. Figure 1 (inset) shows the values of fluorescence intensities at the time necessary to reach equilibrium at pH 8.0 (i.e. 100 s for cryptogein and 300 s for capsicein). The fluorescence intensities of the DHE-cryptogein complex changed notably between pH 4.5 and 5.5 and then remained constant. A similar effect was observed for capsicein despite its different pI and slower kinetics.

The fluorescence enhancement of DHE upon binding to elicitins was used to determine binding parameters of DHE by plotting 1/free DHE *vs.* 1/bound DHE [17].

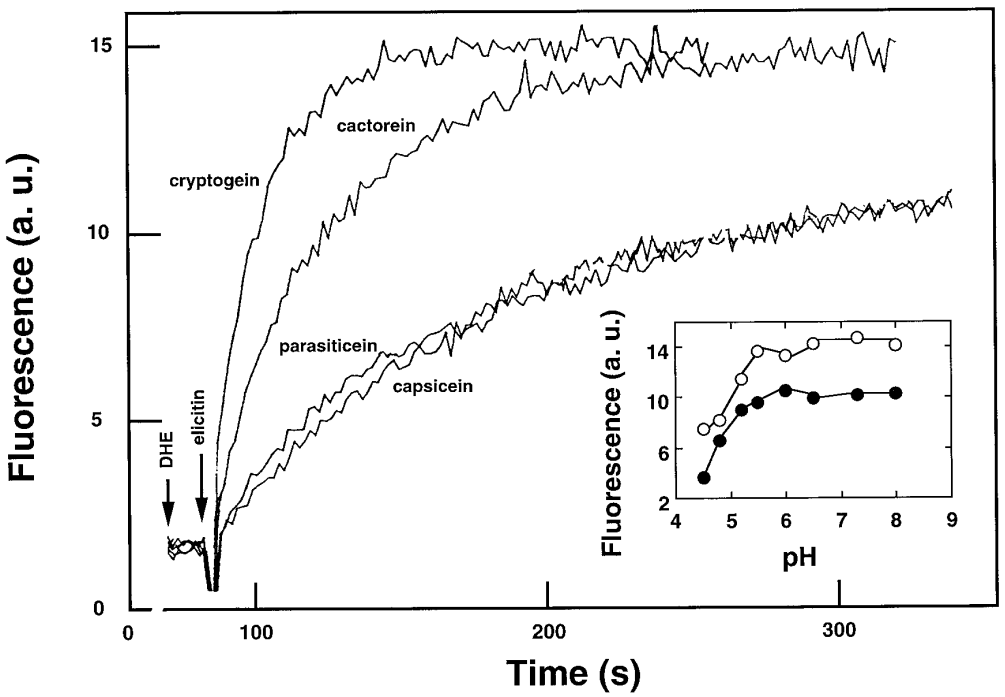

FIG. 1. Kinetics of the elicitin-DHE interactions. DHE (1.26 μM) and elicitin (2 μM) were added in buffer I. Excitation wavelength was set at 325 nm, emission at 370 nm. Inset: Effect of the pH on the interaction of cryptogein (○) and capsicein (●) with DHE, pH set at different values between 4.5 and 8.0 with MES-Tris buffers. The data represent the fluorescence intensities reached 100 s (cryptogein) or 300 s (capsicein) after the addition of the elicitin to DHE.

The results are shown in Table 1. Dissociation constants of the complexes of the five elicitins with DHE were between 0.11 μM (for cactorein) and 0.58 μM (for parasiticein). The stoichiometry of the interaction was shown to be between 0.86 and 1.16 sterol binding site per elicitin molecule. Relative fluorescence intensities of DHE bound to elicitins (Q_b), obtained from the titration of a constant DHE concentration with elicitins, were between 10.3 and 13.2 a.u./μM DHE.

Isolation of a sterol-elicitin complex. In order to confirm the formation of the sterol-elicitin complex shown fluorometrically, we isolated a stigmasterol-cryptogein complex by chromatography on Sephadex G-25. The first eluted fraction contained stigmasterol and cryptogein. The recovery of cryptogein was 98-102% (from two experiments) and the molar ratio of cryptogein/stigmasterol was 1.08-0.93. The excess of sterol adsorbed on Sephadex was then eluted and the total recovery of stigmasterol was 102-114%. These results confirmed the stoichiometry of cryptogein-sterol binding obtained fluorometrically.

Interaction of cryptogein with phytosterols. TNS was used to study the binding of non fluorescent plant sterols to elicitins. Fluorescence of the probe in water is negligible and its hydrophobic interaction with cryptogein gave rise to an increase in fluorescence at 430 nm. The interaction was faster than that of DHE with cryptogein (kinetic curve is not shown). Figure 2 (inset A) shows a fluorescence titration curve of cryptogein with TNS, in the presence or absence of stigmasterol. The plots of 1/bound TNS *vs.* 1/free TNS gave a dissociation constant of 1.45 ± 0.07 μM and 1.4 ± 0.03 (3 experiments) TNS binding sites per cryptogein molecule. So, cryptogein has lower affinity for TNS than for DHE (Table 1).

TABLE 1

Binding Parameters of DHE to Elicitins

Elicitin	K_d (μM)	N (mol/mol)	Q_b (a.u./μM)
Cactorein	0.11 ± 0.02	0.86 ± 0.08	13.2
Capsicein	0.55 ± 0.11	0.85 ± 0.14	11.8
Cryptogein	0.56 ± 0.04	0.90 ± 0.10	12.5
Parasiticein	0.58 ± 0.14	1.16 ± 0.25	10.3

Note. The values of the dissociation constants (K_d) and the numbers of binding sites (N) were calculated on the basis of the titration of 2 μM elicitin with 0.2-3.8 μM DHE in buffer I. The correlation coefficient of binding plots was higher than 0.95. For capsicein and parasiticein, fluorescent intensities used were those obtained 5 min after mixing with DHE. The values of relative fluorescence intensity of bound DHE (Q_b) were obtained from the titration of 0.25 μM DHE with elicitins, until a constant fluorescence level was obtained.

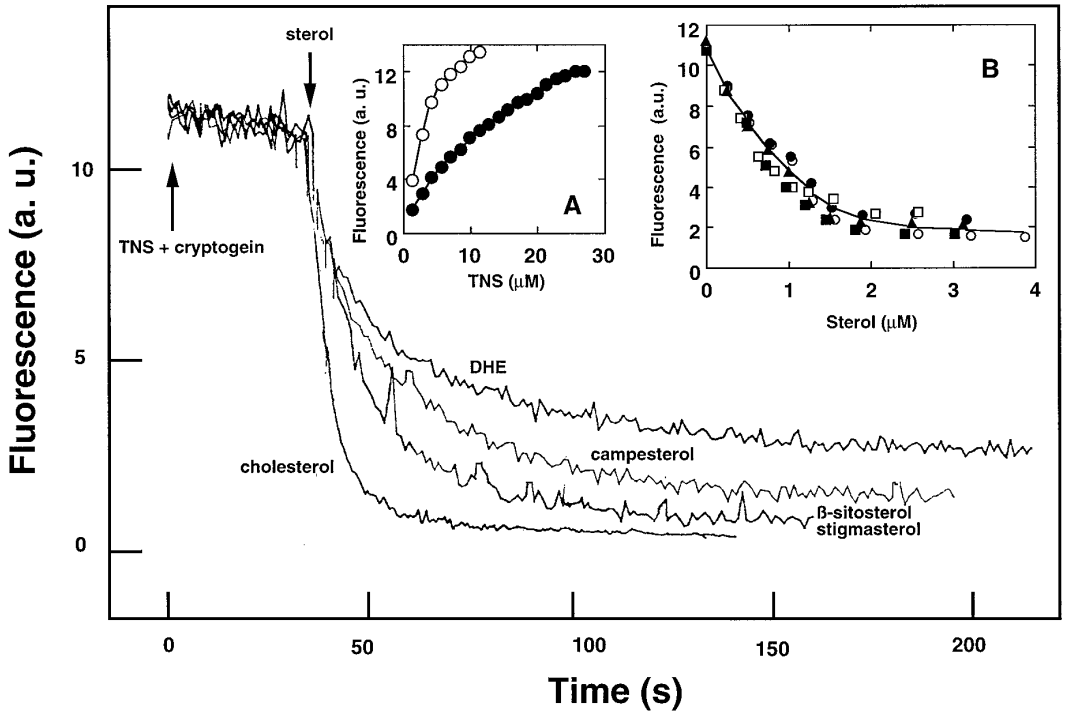

FIG. 2. Kinetic curves of the fluorescence quenching by sterols of TNS bound to cryptogein. TNS (7 μM) was added to cryptogein (2.5 μM) in buffer I and incubated for 2 min. Excitation and emission wavelengths were set at 355 and 430 nm, respectively. Thereafter, sterol (1.2 μM) was added and fluorescence was recorded during 200 s. Inset A: Fluorescence titration curve of cryptogein with TNS. Cryptogein (2.5 μM) (m); cryptogein preincubated for 5 min with stigmasterol (2.5 μM) (●). Inset B: Titration curves of the fluorescence quenching by sterols of TNS bound to cryptogein. The cryptogein-TNS complex was titrated with cholesterol (○), campesterol (▲), β-sitosterol (■), dehydroergosterol (●) and stigmasterol (□) in buffer I. Fluorescence intensity of TNS was read after signal stabilization (2 - 3 min).

The addition of a non fluorescent phytosterol to cryptogein preincubated with TNS induced a large decrease in TNS fluorescence which stabilized after 1 - 3 min. (Fig. 2). The kinetics of the quenching was fast in the case of cholesterol and slower in the case of campesterol, stigmasterol and β-sitosterol or DHE. We did not observe any interaction between sterol and TNS. For example, when cholesterol alone (3 μM) was added to TNS (7 μM), no change in fluorescence was observed. Thus, the quenching of TNS fluorescence cannot be due to a redistribution of TNS between protein and cholesterol micelles, but could result either from a conformation change of cryptogein caused by the binding of the sterol, or a simple displacement of the dye bound to cryptogein by sterol. To test these hypotheses, cryptogein was preincubated with stigmasterol and the titration curve of the complex was compared with that of cryptogein without stigmasterol. The shapes of these titration curves (Fig. 2, inset A) were different whereas maximal fluorescence values obtained in the presence of excess of TNS reached a similar value. This suggests that a competition and the displacement of the dye by sterol due to higher binding constant of sterol is the most probable mechanism of fluorescence quenching of the probe-cryptogein complex.

Figure 2 (inset B) shows a titration curve of the cryptogein-TNS complex with different sterols added to the mixture. Fluorescence was read after signal stabilization. All sterols bound to cryptogein with similar dose-response curves but the fluorescence of TNS could not be totally quenched by any of the sterols.

Elicitin-catalyzed transfer of sterols between artificial membranes. In liposomes, steady-state polarization of fluorescence seems to be a better marker of sterol transfer than fluorescence intensity measurements [6]. The interaction of DHE molecules themselves in liposomes, resulting in radiationless energy transfer, will also decrease fluorescence polarization. The molecular transfer of DHE between liposomes can be visualized by the increase in steady-state polarization of DHE fluorescence in the donor-acceptor mixture [6]. Donor liposomes contained PC, PS and DHE, acceptor liposomes contained PC, PS and cholesterol. The ratio between donor and acceptor liposomes was 1:9. The presence of PS enhances negative surface charge which should stimulate considerably the exchange catalyzed by a basic sterol carrier protein [6]. Due to energy transfer between DHE molecules packed in donor liposomes, fluorescence polarization is low (about 0.21).

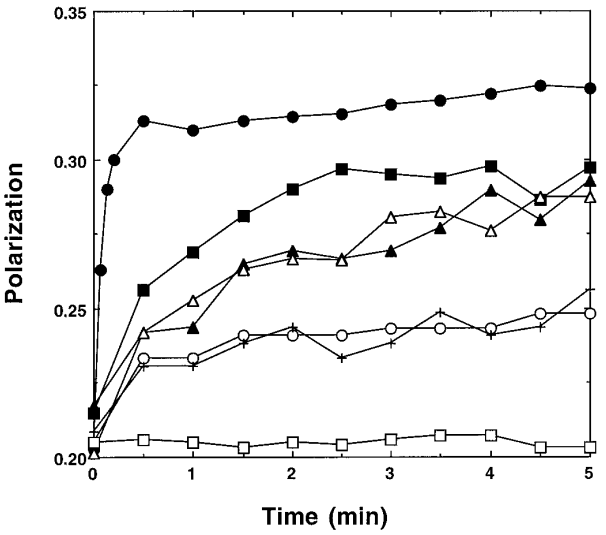

FIG. 3. Elicitin-catalyzed exchange of sterols in SUV. The ratio between donor vesicles containing PC, PS, DHE and acceptor vesicles containing PC, PS, cholesterol was 1:9. The total concentration of vesicles in buffer II was 140 μg/ml and that of elicitin was 1 μM. (□) donor vesicles plus cryptogein, (○) spontaneous transfer; (●) cryptogein; (▲) capsicein; (■) , cactorein; (△, parasiticein; (+) BSA 1 μM.

The changes in polarization upon mixing donor and acceptor SUV, in the absence or presence of elicitins, are shown in Fig. 3. The spontaneous transfer of sterol induced an increase in fluorescence polarization from 0.20 to 0.25. Cryptogein was the most efficient elicitin strongly and rapidly stimulating the sterol exchange compared to the spontaneous exchange, in the absence of elicitin (Fig. 3). Cactorein proved to be less efficient than cryptogein, and the effects of parasiticein and capsicein were weaker but significant. When cryptogein (1 μM) was added to donor vesicles alone (140 μg/ml), no change in polarization was observed. BSA did not catalyze any transfert of sterols between artificial membranes (Fig. 3).

DISCUSSION

Elicitins bind DHE. Modifications of fluorescence emission spectra of DHE upon elicitin addition proved that DHE interacts with a hydrophobic part of these proteins. Since, DHE fluorescence is not very sensitive to solvent polarity [18], the increase in fluorescence intensity and the change of the shape of the emission spectra upon binding of DHE to a hydrophobic part of elicitins could result from a separation of DHE molecules which were concentrated in micelles where energy transfer was considered as a possible mechanism of energy dissipation and fluorescence quenching [4, 6]. This could explain why fluorescence intensities of bound DHE in all elicitins are very close (Table 1). The increase in fluorescence allowed us to determine binding parameters of sterols to elicitins. All elicitins we studied up to now had the same binding stoichiometry, i.e. 1 sterol molecule bound per elicitin molecule, and dissociation constants are in the same range (0.11 − 0.58 μM). The formation and the stoichiometry of the sterol-elicitin complex was confirmed by isolating this complex chromatographically. Several animal intracellular proteins are also able to bind sterols. The sterol carrier protein-2 SCP2 from rat liver binds dehydroergosterol with an apparent dissociation constant of 1.2-1.5 μM and a stoichiometry of 0.8-1.0 sterol per protein molecule [5]. Another structurally different sterol carrier protein, SCP from rat liver, binds dehydroergosterol with a Kd of 0.88 μM and a similar stoichiometry [4]. A rat liver fatty acid binding protein seems to have higher affinity with a K_d of 0.29 μM and 0.9 binding site per protein molecule [5]. On the other hand, BSA has a lower affinity (K_d = 2.9 μM) and 6 to 7 binding sites per molecule of protein [5] and we demonstrated that this protein is unable to catalyze sterol transfert between artificial membranes. These results show that elicitins have similar affinity and number of DHE binding sites to those previously described for sterol binding proteins from mammals. Despite identical binding parameters, kinetics of their interaction with DHE are different, cryptogein and cactorein being the most rapid (Fig. 1).

Primary structures of elicitins display highly conserved domains [20]. Comparison of elicitin sequences with protein databases did not reveal any similarity of significant relevance. Refined analyses were performed by local alignments of elicitins with a subset of sterol carrier proteins (SCP2) from various organisms, i. e. human, chicken, rat, mouse that were retrieved from Genbank database. Here again, the similarity level was too low to be of value (not shown). This is in agreement with previous statements that indicated that elicitin sequences are not related to any known sequence [12]. The crystallographic structure of cryptogein shows that the amino acids forming its hydrophobic pocket are contained in these conserved domains [14]. Therefore, the binding kinetics seem to be influenced by conformational modifications of the proteins rather than by the structures of the amino acids involved in the hydrophobic pocket. Both kinetics of basic cryptogein and acidic parasiticein changed between pH 4.5 and 5.5. This is probably caused by dissociation of glutamic and aspartic acid which are the only acidic dissociable residues in these elicitins [20]. These kinetics prove that, in physiological conditions, i.e. between pH 5.5 and 7.0, the interaction of elicitins with sterols is not pH-dependent whereas, some elicitin-induced effects on tobacco cells are pH-dependent [24].

Elicitins bind phytosterols. TNS interacts non specifically with many proteins and biological membranes

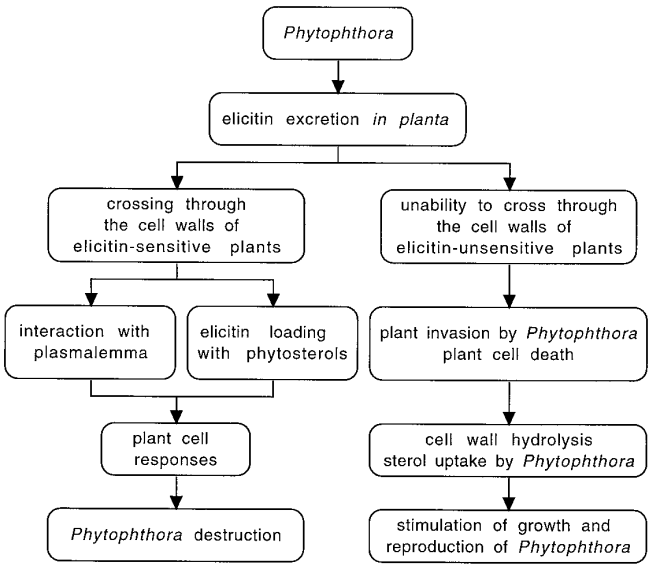

FIG. 4 A working hypothesis illustrating two types of plant-*Phytophthora* interactions, with elicitin-sensitive and elicitin-unsensitive plants. In the first case, elicitin produced *in planta* could interact with plasma membranes. This interaction leads to plant cell responses (hypersensitive reaction) and then to *Phytophthora* destruction. With the elicitin-unsensitive plants, either the elicitin is not excreted *in planta*, or the produced elicitin is unable to cross over cell walls and plant cells do not exhibit any response. Then, the fungus can invade the plant, provokes cell wall hydrolysis allowing sterols to be accessible to elicitins.

and was used to study conformation changes of proteins [25-27]. The results reported here indicated that there is more than 1 TNS binding site per cryptogein molecule. If fluorescence quantum yields of TNS in these sites are not identical, the use of such a simple binding analysis may not be quite relevant. However, fluorescence of TNS bound to cryptogein was used to show that cryptogein could interact with phytosterols. The addition of these sterols to TNS-cryptogein complex caused a decrease in TNS fluorescence. This is probably caused by the displacement of TNS by phytosterols, according to their lower dissociation constants. Titration curves of cryptogein by TNS, in the presence and absence of stigmasterol, seemed to confirm this hypothesis (Fig. 2, inset). The same effect was observed by Stryer [28] who found a competition between hemin and anilinonaphtalenesulfonate bound on apomyoglobin, and quenching of the dye fluorescence by hemin. Since TNS is an amphipatic probe with negative charge, its binding site on cryptogein may be different from that of sterols.

Despite distinct kinetics of sterol-cryptogein interaction (Fig. 2), all the sterols bound to cryptogein with similar affinity. The hydrophobic core of cryptogein similarly interacted with the different sterols studied. Nevertheless, the unability of sterols to totally quench the fluorescence of TNS (even when they are in excess) remains to be explained.

Elicitin-mediated exchange of DHE between liposomes. Fluorescence polarization experiments showed that addition of elicitins, stimulated increase in fluorescence polarization of DHE in donor-acceptor mixture of SUV (Fig. 3). Fluorescence of the elicitin-DHE complex did not contribute to increase the value of fluorescence polarization, because (i) we observed a competition between stigmasterol and DHE for binding to cryptogein, (ii) the ratio DHE:stigmasterol in the experiment was 1:9, so that the complex stigmasterol-cryptogein prevailed and the fluorescence of DHE-cryptogein must be weak.

These changes were explained as a transfer of sterols between donor and acceptor SUV. Monitoring the quenching of fluorescence of dyes entrapped in vesicles, it was shown that SCP-2 did not stimulate fusion of vesicles [7]. Moreover, an inhibition of sterol binding to SCP-2, by modification of SH-groups, inhibited completely these changes in fluorescence polarization so that binding of sterol into hydrophobic pocket had to be implied [7].

Our results demonstrated that elicitins are able to bind sterol molecules and to catalyze a transfer of sterol between artificial membranes. Cryptogein and cactorein are the most potent sterol carrier proteins, whereas capsicein and parasiticein are the least efficient. As suggested by Schroeder *et al.* [6], the ability of a protein to bind sterols does not necessarily allow to conclude that this protein enhance transfer of sterols between membranes. This is confirmed by our finding showing that cryptogein and cactorein are more potent in stimulating sterol transfer than the others, although binding parameters of all elicitins are similar. Moreover, kinetics of sterol binding to BSA is very fast [17] and the unability of BSA to stimulate sterol transfer is shown here. So, rapid kinetics of sterol binding to proteins are not necessarily related to a more efficient sterol transfer activity. In this paper, we demonstrated that elicitins are both sterol binding and carrier proteins. This is the first evidence for such an activity associated to acidic and basic extracellular proteins. However, the most efficient transfer activity is related to the basic protein of this family.

We previously studied the response of *Nicotiana tabacum* to elicitins. These proteins induce necroses in tobacco plants which then become resistant to tobacco pathogens [20, 29]. In tobacco cell suspensions treated with cryptogein, the earliest events reported are a binding to a specific high-affinity protein on the plasmalemma, an alkalization of the extracellular medium, ions exchanges, and a transient production of active oxygen species [24]. These responses are blocked by protein kinase inhibitors showing that phosphorylation steps are implied in the initial transduction of the elic-

itation process [24]. The plasma membrane is probably the target for the first interaction between tobacco cells and cryptogein [30], but nothing is known about the molecular mechanisms involved in this initial interaction. Experiments in progress demonstrate that elicitins are able to pick up phytosterols from tobacco cell suspensions or from purified tobacco plasmalemma. From these results, elicitins appear to be able to migrate, through cell wall, into the plasma membranes of tobacco cells.

Therefore, elicitins act as inducers of a hypersensitive-like response in tobacco during incompatible interactions, and they also display a sterol carrier protein activity. In order to understand the role played by elicitins in plant-*Phytophthora* interaction, we propose a working hypothesis where these two properties are integrated (Fig. 4). This scheme shows that the ability of elicitins to pass through the cell walls could be a crucial step in this plant-fungus interaction. Further experiments should clarify this possibility.

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