Biochimica et Biophysica Acta 812 (1985) 633–642
Elsevier

BBA 72382

Effects of triazoles on fungi. III. Composition of a plasma membrane-enriched fraction of *Taphrina deformans* *

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(Received June 19th, 1984)

Key words: Triazole; Sterol/phospholipid ratio; Methyl sterol; (Plasma membrane, *T. deformans*)

Comparative chemical analyses were conducted with plasma membrane-enriched fractions of *Taphrina deformans* cells grown in a medium with or without the C-14 demethylation inhibitor propiconazole at a concentration that gives 50% growth inhibition. The membrane fractions were prepared using differential and discontinuous sucrose density gradient centrifugation, and characterized by cytochemical, enzymatic and chemical analyses. Membranes of nontreated cells were similar to those from other fungi with a protein/lipid ratio of 1.2, 13% phospholipid content in the membrane lipid (122 μg/mg protein), and a relatively high sterol/phospholipid molar ratio of 0.69. The corresponding membrane fraction from propiconazole-treated cells had 24% less lipid, 27% less phospholipid, 5-times more triacylglycerol relative to other neutral acyl lipids, and over a 2-fold higher sterol/phospholipid ratio. The greater sterol/phospholipid ratio was due to a higher C-14 methyl sterol content rather than less functional sterol (brassicasterol). Membranes from treated cells contained slightly less protein than those from nontreated cells, but there was little difference in the electrophoretic separation patterns of solubilized membrane polypeptides.

Introduction

In recent years, several pyridine, pyrimidine, morpholine, piperazine and triazole derivatives have been found to be effective fungal growth inhibitors. In spite of their chemical diversity, these substances have a common site of action in that they block one of the reactions in the oxidative removal of the C-14 α-methyl group which is lost in the conversion of lanosterol to primary functional sterols such as ergosterol [1]. The triazole propiconazole used in this study, like its ethyl homologue ectaconazole [2,3], is such an inhibitor [4]. Although there are other responses to these inhibitors involving lipid metabolism [4], C-14 demethylation inhibition is generally considered to be the primary biochemical activity of these substances resulting in growth inhibition. If indeed this is true, it must be assumed that some growth-limiting process is negatively affected, either directly or indirectly, by a reduction in the functional sterol level, and/or perhaps the accumulation of C-14 methyl sterols that are generally believed to be nonfunctional.

As sterols are generally considered to exert their

* Alabama Agricultural Experiment Station Publication No. 6-84618.

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; LysoPC, lysophosphatidylcholine.

primary biological activity in membranes [5], we have proposed [4] that growth inhibition by sublethal doses of C-14 demethylation inhibitors may result from changes in plasma membrane chemistry, and hence function, for the following reasons: (a) the relatively high sterol/phospholipid ratios generally believed to be characteristic of plasma membranes would be expected to have functional significance; (b) C-14 demethylation inhibitors generally do not significantly inhibit respiration [6], suggesting that mitochondrial membranes are not involved in their primary growth inhibitory action; (c) cell-permeability changes resulting from treatment with sublethal doses of propiconazole are not due to direct interaction of the inhibitor with the plasma membrane, but instead are the result of growth (metabolic) related changes in this membrane due to inhibitor treatment [7]; (d) spore germination is not inhibited by C-14 demethylation inhibitors, but incubation of spores in a medium containing such an inhibitor leads to amorphous growth (unpublished data), indicating that cell-wall composition is altered and possible plasma membrane involvement.

We chemically analyzed a plasma membrane-enriched fraction from C-14 demethylation inhibitor-treated *Taphrina deformans* cells with the objectives of characterizing this membrane from a species not previously studied in this regard, and to determine whether such treatment of cells results in chemical alterations of this membrane that may explain, at least in part, how blocking C-14 demethylation is translated to growth inhibition. *T. deformans* is a pathogen of peach (*Prunus persica* (L) Batsch) that grows as the yeast-form in laboratory culture. Preliminary accounts of this work were presented as posters at the 24th International Conference on the Biochemistry of Lipids in Toulouse, France (1983) and at the 6th International Symposium on the Structure, Function, and Metabolism of Plant Lipids, Neuchatel, Switzerland (1984).

Experimental procedures

Fungal culture. T. deformans was grown for 80 h on a culture medium containing 1% yeast extract (Difco) and 2% glucose (YG medium), at pH 5.4. The medium was inoculated with 72-h cultures and incubated on an alternative shaker (76 cycles/min) at 18°C.

Isolation and characterization of the plasma membrane-enriched fraction. Cells were ground using a Braun homogenizer with 0.17–0.18 mm glass beads. Isolation of the plasma membranes from the homogenate was conducted as previously described by Rami et al. [8] with the following modifications: the homogenization medium consisted of 0.25 M sucrose, 50 mM Tris-HCl buffer (pH 7.4), 1 mM EDTA, 0.1 mM $MgCl_2$, chloramphenicol (200 mg/l) and cycloheximide (200 mg/l). The homogenate was fractionated through a series of differential and discontinuous sucrose density gradient centrifugations. Material at the sucrose density interfaces was collected with a Pasteur pipet.

NADH and NADPH cytochrome *c* reductases were assayed according to Hodges and Leonard [9]. The former enzyme was assayed in the presence of antimycin A (14 μg/ml). Succinate dehydrogenase activity was determined by measuring 2,6-dichlorophenolindophenol reduction as described by Marriott [10]. Mg^{2+}-ATPase was assayed in the presence of oligomycin (6.65 μg/ml) [9]. AMPase and IDPase activities were determined according to Morre [11] and Morre et al. [12]. Phosphatase reactions were stopped with 10% trichloroacetic acid and, after centrifugation at $4000 \times g$ for 10 min, inorganic phosphate in the supernatant was measured as described by Fiske and SubbaRow [13].

Extraction of lipids. Lipid was extracted from the membranes essentially by the technique of Bligh and Dyer [15]. Lyophilized membrane samples (25–100 mg) were washed twice with 30 ml $CHCl_3$/MeOH (1 : 1, v/v) containing 0.5% H_2O for 1 h in a water-bath at 40°C with occasional manual stirring. Insoluble material was separated by filtration through a glass-fiber filter (Whatman GF/A), and the organic phase was washed with 6% NaCl prior to drying with anhydrous Na_2SO_4 and evaporating the solvent under a stream of N_2.

Chromatography of lipids. Thin-layer (TLC) and gas-liquid chromatographic (GLC) analyses of the lipids were conducted as described previously by Weete et al. [4]. Sterols obtained in the non-saponifiable fraction of the total membrane lipid alkaline hydrolysate (see below) were quantitated

by GLC using ergosterol as the standard. Percentages of the individual sterols were based on relative areas under each peak determined by triangulation ($A = HW_{1/2H}$).

Miscellaneous methods. Proteins and lipid phosphorus were analyzed by the method of Lowry et al. [14] and Bartlett [16], respectively. Acyl lipids were hydrolyzed in ethanolic KOH as described by Kates [17] and fatty acid methyl ester derivatives were then prepared using BF_3 in methanol.

Electrophoresis. Dry membrane samples were dissolved (6 mg/ml) in 0.062 M Tris-HCl buffer (pH 9.3) containing 2% sodium dodecyl sulfate (SDS), 10% glycerol and 5% 2-mercaptoethanol. Each sample was heated for 1–2 min at 100°C prior to analysis. SDS-polyacrylamide gel electrophoresis of the samples was conducted according to Laemmli [18] using a 7–12% linear gradient of acrylamide in a 1.5 mm thick slab gel. 10 and 20 μl of each sample were electrophoresed, gels were stained with 0.1% Coomassie brilliant blue in 50% (w/v) trichloroacetic acid, and absorbance traces were performed on each stained sample lane at 560 nm using a Gilford Spectrophotometer (Model 2600).

Electron microscopy. The membrane fractions were resuspended with the initial buffer and centrifuged at 25 000 × *g* for 20 min. A part of the pellet was resuspended in the buffer without sucrose and fixed with glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) (final percentage of glutaraldehyde = 2.5%) for 2 h. After centrifugation at 4500 × *g*, the pellet was postfixed with 1% OsO_4 for 12 h.

Cytochemical techniques. The cytochemical technique for the detection of polysaccharides as established by Thiery [19] was performed on a gold grid as described by Dargent and Touze'-Soulet [20]. Control sections demonstrating the specificity of the reaction were obtained by withholding the thiocarbohydrazide treatment or by oxidation with hydrogen peroxide.

Materials. Propiconazole was a gift from Dr. Homer M. LaBaron of the Ciba Geigy Corporation, Greensboro, NC. Lipid standards and BF_3 in methanol were obtained from Applied Science Laboratories (phospholipids, lanosterol and fatty acid methyl esters) and Sigma Chemical Co. (ergosterol).

Results

Characterization of the plasma membranes from nontreated cells

After removing the heavier membranes by differential centrifugation, the pellet obtained at 55 000 × *g* was separated into four fractions on a discontinuous sucrose density gradient. Succinate dehydrogenase activity was low in each fraction (Table I), indicating little contamination by mitochondrial membranes. Excluding fraction 3 (F3), which contained relatively little particulate material, but had the highest activities of the enzyme markers, both fractions 5 (F5) and 6 (F6) exhibited relatively high oligomycin-insensitive Mg^{2+}-ATPase activity at pH 6.5; low NADH (antimycin A-insensitive) and NADPH cytochrome *c* reductase activities in these fractions indicated that there was little contamination by endoplasmic reticulum. AMPase activity was detected in F5. It was concluded that F5 and F6 were enriched in plasma membrane, but because of the relatively high IDPase activity they were probably contaminated with Golgi-like membranes. F5 was visually the largest fraction and selected for further study. It contained regularly shaped vesicles ranging from 1000 to 5000 Å in diameter (Fig. 1D and E).

The protein/lipid ratio of the membranes in F5 was 1.2, with lipid comprising 42% of the dry weight (Table II). The calculated phospholipid content (based on lipid phosphorus) of the membranes was 13% of the total lipid and 6% of the membrane dry weight. The phospholipid content of the membranes based on protein content was 122 μg/mg. Based on densitometric scans of TLC plates visualized by charring with sulfuric acid, polar lipid (phospholipid plus unidentified polar lipid) comprised about 29.6% of the total (Table III, Fig. 2). Otherwise, free fatty acids were the major lipids in the membranes at 28.4%, followed by unknown No. 1 (10.3%), diacylglycerols (11.6%), triacylglycerols (9.0%) and steryl esters (5.2%). With the exception of the diacylglycerol fraction, where it comprised 40% of the total, oleic acid ($C_{18:1}$) was the major fatty component of the acyl lipids, ranging from 51 to 59% (Table IV).

Sterols represented about 6% of the lipid from the *Taphrina* plasma membrane-enriched fraction,

TABLE I

ENZYME ACTIVITIES IN THE CRUDE HOMOGENATE AND MEMBRANOUS FRACTIONS OF *T. deformans*

C, nontreated cells; P, propiconazole-treated cells.

Cell fractions	Succinate dehydrogenase [a]		NADH-cytochrome *c* reductase [a,b]		NADPH-cytochrome reductase [a]		Mg^{2+}-ATPase [c]		AMPase [c]		IDPase [c]	
	C	P	C	P	C	P	C	P	C	P	C	P
Homogenate	4.36	9.77	5.14	13.72	0.99	0.84	33.98	26.68	1.69	3.52	21.80	21.41
F3	0	0	5.83	11.42	3.51	0.95	97.90	40.31	4.60	trace	20.10	29.06
F5	0.58	0.65	3.29	3.35	1.37	0.31	38.00	39.28	3.93	trace	20.49	27.79
F6	0.63	0.38	2.85	4.82	0.29	0.36	22.33	28.26	0	trace	15.85	21.01
F7	0.88	0.62	1.68	1.76	0	0.14	11.83	32.57	0	0	8.88	17.97

[a] μmol/min per mg protein.
[b] Plus antimycin A.
[c] μm P_i/h per mg protein.

TABLE IV

FATTY ACID COMPOSITION OF LIPIDS OF PLASMA MEMBRANE FRACTIONS OF PROPICONAZOLE-TREATED (P) AND NONTREATED CELLS (C) OF *T. deformans*

Fatty acid	Composition (%)											
	total lipid		polar lipid		monoacyl-glycerol		diacyl-glycerol		free fatty acid		triacyl-glycerol	
	C	P	C	P	C	P	C	P	C	P	C	P
$C_{16:0}$	8.0±1.3	19.1±3.2	18.5±2.8	23.7±0.4	18.4±0.0	23.3±6.1	26.0±3.0	16.2±1.3	9.9±2.6	24.1±1.5	15.1±1.8	18.1±4.7
$C_{18:0}$	8.6±0.9	25.0±4.5	13.5±1.8	12.6±0.5	15.4±3.4	19.4±0.8	19.2±1.5	16.8±0.5	5.0±0.2	18.0±2.9	10.3±0.3	25.8±0.5
$C_{18:1}$	60.1±0.4	20.6±3.3	51.0±4.4	11.9±0.2	51.0±1.8	28.2±1.9	39.7±3.3	30.6±2.5	59.4±1.0	16.7±4.0	56.4±3.4	29.7±1.6
$C_{18:2}$	22.3±3.5	27.6±6.5	15.8±0.4	39.2±0.9	14.1±1.1	22.9±2.6	13.0±1.5	27.7±0.1	24.0±3.2	31.5±0.9	16.7±1.7	21.6±4.2
$C_{18:3}$	1.7±0.6	7.6±2.5	1.2±0.0	12.9±0.2	1.1±0.6	6.4±0.8	1.7±0.1	8.8±0.9	1.7±0.6	10.9±1.9	1.5±0.5	4.9±1.7
Δ/mole [a]	1.10	0.99	0.86	1.29	0.83	0.93	0.71	1.13	1.13	1.12	0.94	0.87
S/U [b]	0.20	0.79	0.47	0.54	0.51	0.74	0.85	0.55	0.18	0.71	0.34	0.78
M/(D+T) [c]	2.50	0.59	3.00	0.23	3.36	0.96	2.70	0.84	2.31	0.39	3.10	1.12

[a] Δ/mole = ((%monoenes)+2(%dienes)+3(%trienes))/100.
[b] S/U = Σ% saturated/Σ% unsaturated fatty acids.
[c] M/D+T = $\%C_{18:1}/\%(C_{18:2}+C_{18:3})$; M = monoenes, D = dienes, T = trienes.

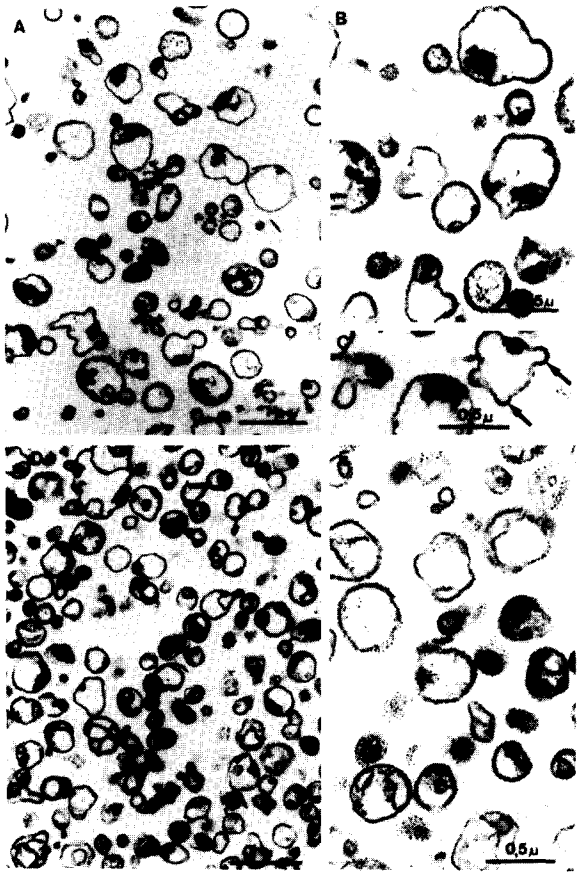

Fig. 1. Electron micrographs of a plasma membrane-enriched fraction (F5), from *T. deformans* after the Thiery test [19]. (A) Propiconazole-treated (15000×); (B–C) propiconazole-treated showing characteristic protuberances (arrows) (30000×); (D) control (15000×); (E) control (30000×).

TABLE II

LIPID AND PROTEIN CONTENTS OF PLASMA MEMBRANE-ENRICHED FRACTIONS OF PROPICONAZOLE- AND NONTREATED CELLS OF *T. deformans*

Component	Control	Treated
Protein/lipid (w/w)	1.20	0.98
Lipid (% dry wt.)	42.4±1.1	51.7±0.3
Phospholipid		
(% total lipid)	13.4±1.3	8.6±0.5
(μg/ml protein)	121.8±0.1	89.2±8.9
LysoPC (% phospholipid)	7.1±1.1	9.1±0.7
PC	19.6±3.2	22.0±0.9
PS+PI	13.4±1.0	15.9±1.2
PE	31.0±4.9	28.5±2.3
Phosphatidic acid	19.0±2.6	14.9±0.1
Unknown at solvent front	9.9±2.0	9.6±1.9

TABLE III

LIPID COMPOSITION OF A PLASMA MEMBRANE-ENRICHED FRACTION OF *T. deformans*

Values were calculated from integrator printouts on recordings of densitometric scans of TLC plates sprayed with 30% H_2SO_4 and heated at 125°C for 30 min. Since the color of sterols on the plates was different from the other lipids, a more accurate estimate of sterol content was made using GLC, (see Experimental procedures) and other integrator values were normalized accordingly in the calculation of relative proportions. The values obtained using this method were in line with those calculated from fatty acid analyses of individual lipids isolated by TLC and using the methyl ester of $C_{17:0}$ as an internal standard. The data in this sample are from only one replication of each treatment.

Lipid	Composition (%)	
	control	propiconazole-treated
Polar lipid [a]	29.6	20.5
Unknown No. 1	10.3	11.9
Diacylglycerols	11.6	14.0
Desmethyl sterols	5.5	4.0
4,14-Methyl sterols	–	4.1
Free fatty acids	28.4	25.9
Triacylglycerols	9.0	12.9
Steryl esters	5.2	5.4

[a] Phospholipid plus unidentified polar lipid.

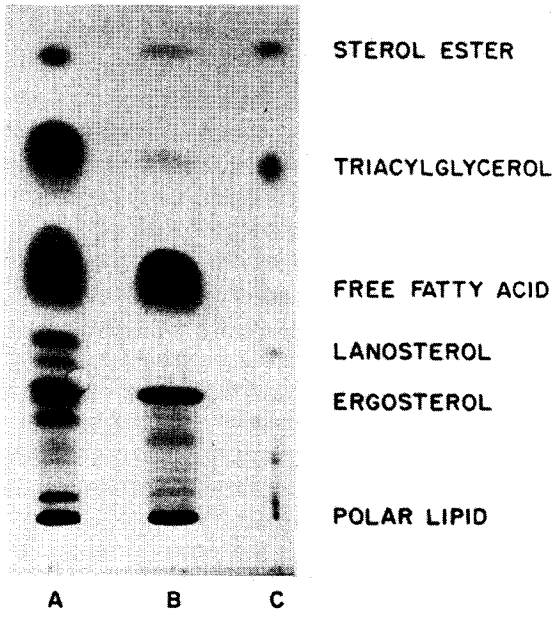

Fig. 2. TLC separation of neutral lipids from a plasma membrane-enriched fraction of propiconazole-treated (A) and nontreated (B) cells of *T. deformans*. Lane C contains lipid standards as labeled. (The plate was developed twice in hexane/diethyl ether/acetic acid (79:20:4, v/v).

92% of which was brassicasterol (Table V, Fig. 3). The sterol/phospholipid molar ratio of these membranes was 0.69 (Table V).

Electrophoretic separation of the SDS-solubilized polypeptides of the *T. deformans* plasma membrane fraction resulted in about 30 bands (Fig. 4). The pattern was similar to that of a plasma membrane preparation from *S. cerevisiae* [31]. Three major size classes of polypeptides with apparent molecular masses of 90–95, 60–62 and 29–40 kDa were visualized in the gel, each displaying a prominent pair of bands. Like the yeast plasma membrane, one of the four major polypeptides of the *Taphrina* plasma membrane fraction had an apparent molecular mass of about 30 kDa.

Plasma membrane fraction from propiconazole-treated cells

Growth of *T. deformans* in a medium containing 0.073 μg/ml of the C-14 demethylation inhibitor propiconazole resulted in about 50% growth inhibition on a dry weight basis [7]. By comparison with F5 membranes from nontreated cells, the membranes and vesicles from the corresponding fraction of propiconazole-treated cells exhibited some characteristic features. For example, the vesicles showed a wider diameter range (700–7300 Å) and possessed protuberances as shown in Fig. 1A–C. Also, the membranes in this fraction did not exhibit uniform staining with silver proteinate as did the corresponding membranes from control cells.

The protein/lipid ratio of these membranes was about 18% less than that of the controls, which was due mainly to the 23% higher lipid

TABLE V

STEROL CONTENT OF *T. deformans* PLASMA MEMBRANES

Component	Control	Propiconazole-treated
Sterols [a]		
(μg/ml dry wt.)	23.2 ± 1.8	40.8 ± 1.1
Brassicasterol		
(μg/mg dry wt.)	23.4 ± 1.8	19.7 ± 2.2
(μg/ml protein)	42.2 ± 8.5	82.9 ± 11.9
(% total lipid)	5.5 ± 0.4	8.1 ± 0.1
Sterol/phospholipid (molar ratio)		
Total sterol [a]/ phospholipid	0.69	1.72
Brassicasterol/ phospholipid	0.63	0.83

[a] Refers to sum of the individual sterols.

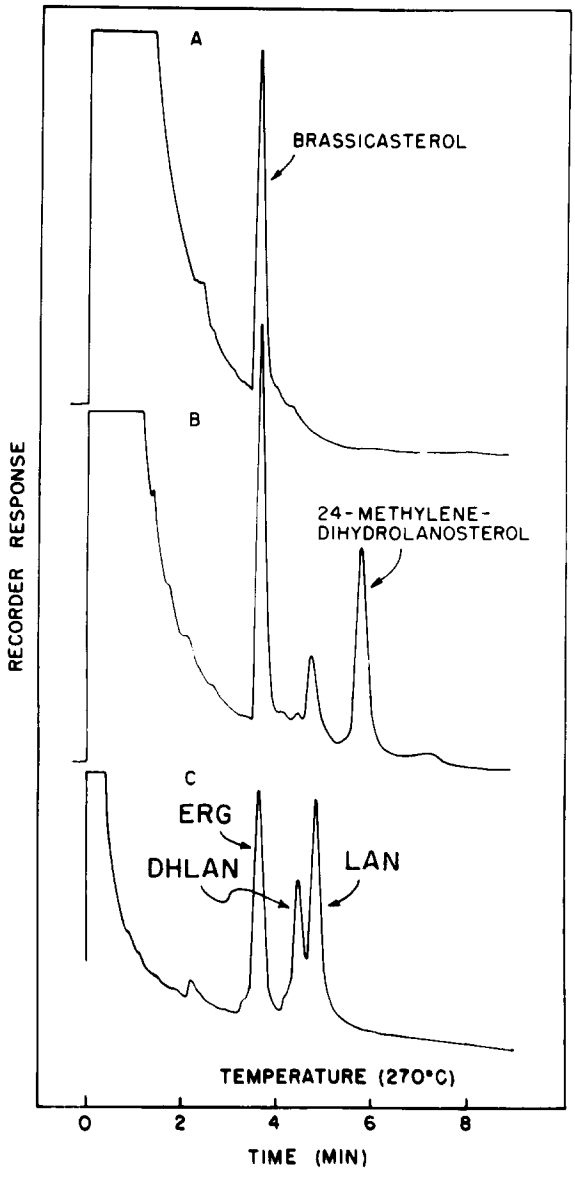

Fig. 3. GLC chromatogram showing separation of total sterols from a plasma membrane-enriched fraction of nontreated (A), propiconazole-treated (B) cells of *T. deformans* and (C) standards. The sterols were separated using a 3 m×2 mm glass column packed with 3% SE-30 on Gas chrome Q. ERG, ergosterol; LAN, lanosterol; DHLAN, dihydrolanosterol.

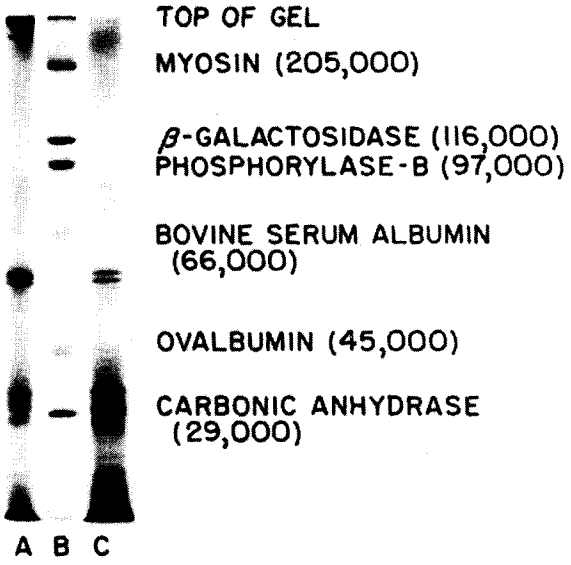

Fig. 4. SDS-gel electrophoretic separation of polypeptides solubilized from plasma membrane enriched fractions from propiconazole-treated (A) and nontreated (C) cells of *T. deformans*. B, molecular mass markers.

content of the membranes from inhibitor-treated cells (Table II). In spite of this, there was 27% (based on protein) less phospholipid in these membranes than in controls. This appeared to be an overall reduction in phospholipid rather than in a specific phospholipid(s), as the relative proportions of the four major types of phospholipids of membranes from treated and nontreated cells were very similar (Table II). The greater lipid content of the membranes from treated cells was due to a higher triacylglycerol content compared to that of controls (Table III and Fig. 2); this was not observed in the total cellular lipid of similarly treated cells [4].

Analysis of component fatty acids by GLC of the total lipid and individual lipid classes indicated that changes in the degree of unsaturation resulted from propiconazole treatment. An increase in the degree of unsaturation (Δ/mole) was noted in the mono- and diacylglycerol fractions, and particularly in the polar lipid (Table IV). Accordingly, the $C_{18:1}/C_{18:2} + C_{18:3}$ ratio was considerably reduced in all the lipid fractions, again particularly in the polar lipid, which was due both to decreased proportions of $C_{18:1}$ and increased $C_{18:2}$ and $C_{18:3}$. Nevertheless, with the exception of diacylglycerols, the saturated/unsaturated ratio was higher in membranes from inhibitor-treated cells compared to those from controls, indicating that there was an overall increase in saturated fatty acids of these membranes.

The plasma membrane-enriched fraction from *T. deformans* cells grown in a medium containing propiconazole at a level that permits only 50% growth relative to controls contained about twice the amount of sterols as the plasma membrane fraction from the untreated cells (Table V). The predominant sterol of the membranes from nontreated cells migrates on a TLC plate as a single band (Fig. 2B); the effects of the inhibitor on sterol metabolism are apparent with the presence of two additional bands with higher R_f values (Fig. 2A). One band, just above that of brassicasterol, is in the region where 4,14-dimethyl sterols would be expected to migrate, and the next band has migration properties similar to those of lanosterol (4α,4β-14α-trimethyl sterol). The latter band contains predominantly 24-methylenedihydrolanosterol, and represents about 31% of the total sterols (Fig. 3). This component of the membrane lipid from treated cells has the same retention time as that from the total lipid of similarly treated *Taphrina* cells in which the expected molecular ion was observed by mass spectrometry [4]. The third major peak is probably obtusifoliol (12%). The molar ratios of both total sterols and brassicasterol to phospholipid were higher in the membranes from propiconazole-treated cells compared to those of the controls (Table V). For brassicasterol/phospholipid, the higher ratio was due almost entirely to the decrease in phospholipid since there was little difference in the brassicasterol content of the membranes. However, in addition to the decrease in phospholipid, the higher total sterol/phospholipid molar ratio was due to a doubling of the sterol content, essentially all of which was due to C-14 methyl sterols.

Only minor differences were observed in the gels containing polypeptides from membranes of inhibitor and nontreated *Taphrina* cells (Fig. 4), suggesting that alteration of the protein composition of the membranes did not contribute appreciably to growth inhibition of *Taphrina* cells by propiconazole.

Discussion

The combined results of electron microscopic, cytochemical, enzymatic and chemical analyses suggest that membrane fraction 5 isolated from *T. deformans* by differential and sucrose density gradient centrifugation is enriched in plasma membrane. The observed membrane diameters, a positive reaction to the Thiery test [19] and relatively high activity of oligomycin-insensitive Mg^{2+}-ATPase support this conclusion [10,21–25]. Relatively high IDPase activity suggests contamination by Golgi-like membranes, but other enzyme markers indicated that F5 is not contaminated with mitochondrial or endoplasmic reticulum membranes.

The protein/lipid ratio of 1.2 for the membranes in F5 is in the range of the values (0.96–2.1) reported for plasma membrane preparations from other fungi [10,26–29] and those reported for a variety of organisms [30]. The phospholipid content of the *Taphrina* membranes is lower than might generally be expected for biological membranes, but is consistent with that of plasma membranes isolated from other fungi such as *Saccharomyces cerevisiae* [26] that contains only 6.4% (of total lipid) phospholipid. Also, plasma membrane lipid from *Candida albicans* yeast protoplasts contain only 16.3% phospholipid or 7% on a membrane dry weight basis [10]. When expressed on the basis of protein, the phospholipid content (122 μg/mg) of *T. deformans* membranes in F5 is at the lower end of the range (127–360 μg/mg) of values reported for plasma membranes from other fungi [10,27,31–33]. There is recent evidence that a low phospholipid content of plasma membranes may be a feature of low temperature adaptation in fungi. For example, low temperature (15°C) acclimation in a slime mutant of *Neurospora crassa* is accompanied by a reduction of phospholipid and an increase of nonphosphorus glycosphingolipids in the plasma membrane [34]. This may in part account for the low phospholipid content of plasma membranes from *T. deformans* which has a relatively low optimum growth temperature of 18°C. Another polar lipid was present but not analyzed in this study.

Whether the neutral acyl lipid is a natural constituent of the *Taphrina* membranes, perhaps compensating at least in part for the relatively low phospholipid content, is not certain. It is conceivable that some of this lipid may have been trapped during preparation of the membranes but if this is so the protein/lipid ratio would be expected to be lower than that observed, and perhaps outside the range expected for fungal plasma membranes (Table II). Also, differences in the proportions of neutral acyl lipid of the membranes from propiconazole-treated cells are not the same as those in the total cellular lipid [4], further suggesting that cellular neutral acyl lipid is not mixed substantially with the membranes (see below). Neutral acyl lipids have been reported as components of plasma membrane preparations from several other fungi [10,26,27]. For example, the plasma membrane fraction from *S. cerevisiae* contains a relatively high amount of free fatty acids [26] as does that from *Taphrina*. The total lipid from *Taphrina* contains a relatively low amount (5%) of free fatty acids [4].

The individual phospholipid composition of the plasma membrane-enriched fraction of *T. deformans* homogenates is qualitatively similar to that previously reported for the total lipid of this strain [4]. As in a slime mutant of *N. crassa* [34] and *S. cerevisiae* NCYC 366 [27], the PC/PE ratio of the *Taphrina* membrane lipid is lower than that of the total cellular lipid. Phosphatidic acid enrichment has been reported for the plasma membranes from *N. crassa* [34], *S. cerevisiae* H 1022 [26] and *Taphrina* (this study) but not for *S. cerevisiae* NCYC 366 [27].

A relatively high sterol/phospholipid molar ratio has been used as a criterion for plasma membrane enrichment in microsomal membrane fractions from chicken-embryo fibroblasts [35]. Although a wide range of sterol/phospholipid ratios have been reported for fungal plasma membrane preparations, relatively few comparisons of the sterol compositions of different membrane fractions of the same cell type have been made. The values for this ratio in plasma membranes from several fungal sources range from 0.77 to 1.3 [26,29,33,34], but lower values have been reported, and 8.97 has been reported for a *S. cerevisiae* strain [26]. Holtz et al. [29] have reported a 3-fold enrichment (0.28–0.77) of sterol relative to phospholipid in the plasma membranes of *Agaricus*

bisporus compared with other membrane fractions. Similar results have been reported for a *N. crassa* strain grown at 37°C. For example, the sterol/phospholipid molar ratio of mitochondria is 0.08, that for the microsomes is 0.5 [36], whereas that of the plasma membrane is 0.81 [34]. Therefore, the value of 0.69 is consistent with the conclusion that F5 from the *Taphrina* cell homogenate is enriched in plasma membranes.

The major responses to sublethal doses of propiconazole by fungi appear to be restricted to lipid metabolism but they are nevertheless complex, probably interelated, and most likely they collectively contribute to a reduction in growth potential of the treated organism. These responses as they occur in *Taphrina* treated with propiconazole are [4]: (a) increase in total lipid; (b) increase in saturated fatty acids; (c) increase in the degree of fatty acid unsaturation ($C_{18:1} \rightarrow C_{C18:2} + C_{18:3}$), particularly in the phospholipid; (d) changes in the relative proportions of various lipids, particularly an increase in the free fatty acid content; (e) increase in total sterol content; and (f) accumulation of C-14α-methyl sterols. With some exceptions, these responses are typical of most fungi treated with the variety of inhibitors that block the C-14 demethylation of lanosterol [2,3,37–39] and, as shown in this study, they occur in a plasma membrane-enriched fraction. This is consistent with our belief that growth inhibition by C-14 demethylation inhibitors may occur at least in part as a result of the alteration of membrane composition. Aside from C-14 demethylation inhibition, it has not been established unequivocally whether these responses are due to direct inhibitor action, or if they are adaptive responses occurring indirectly as a result of alteration of membrane sterol composition.

The suggestion that inhibition of C-14 demethylation is the primary growth inhibitory action of such inhibitors is based mainly on rough time-course correlations between C-14 demethylation and growth inhibition, both of which occur 0.5–4 h after treatment with one of the inhibitors (e.g., miconazole, propiconazole) [2,7,36,37]. If this is true, the question arises as to whether growth inhibition results from an insufficient amount of functional sterol, accumulation of C-14 methyl sterols, or perhaps a combination of both responses to treatment with such an inhibitor. While insufficient functional sterol may contribute to growth inhibition at high inhibitor concentrations, this may not be true at sublethal doses, at least for propiconazole-treated *Taphrina* cells. This is based on the result reported here that plasma membranes from inhibitor-treated *Taphrina* cells contain essentially the same amount of brassicasterol as those from nontreated cells. Furthermore, this may explain the inability to reverse growth reduction of *Taphrina* cells treated with sublethal doses of propiconazole with ergosterol [7] which has also been reported for other fungi treated with different C-14 demethylation inhibitors [37,39,40]. However, it cannot be ruled out that ergosterol is not taken up by cells treated with propiconazole, since it is known that yeast takes up ergosterol from the medium when grown anaerobically but not under aerobic conditions when it is capable of producing sterols.

Although there are some exceptions, there is considerable evidence that the presence of a C-14 α-methyl group in the ring system renders the sterol nonfunctional in eukaryotic systems. For example, Nes et al. [41] have shown that lanosterol or 24-dihydolanosterol will not satisfy the sterol requirement of *S. cerevisiae* under anaerobic conditions, whereas several 14-demethyl sterols in the growth medium permit growth to varying degrees, with ergosterol and brassicasterol being best among those tested. Furthermore, lanosterol incorporated into liposomes composed of PC has no effect on the leakage of glucose from the vesicles, whereas cholesterol reduces leakage [42]. Therefore, growth reduction and metabolism-dependent permeability changes in *Taphrina* treated with sublethal doses of propiconazole [7] may be due mainly to the accumulation of C-14 methyl sterols, even though sufficient functional sterol (brassicasterol) is present.

On the other hand, since lanosterol does not alter the microviscosity of artificial phosphatidylcholine vesicles [42] and is relatively mobile in lipid bilayers [43], it may be that C-14 methyl sterols along with sufficient functional sterol are relatively inocuous in the plasma membranes, and that growth inhibition is due to some activity of the inhibitors other then C-14 demethylation inhibition. This has been suggested recently by

Taylor et al. [44] who have shown that growth inhibition by miconazole and ketoconazole is as effective against a *Saccharomyces* sterol auxotroph grown in medium containing ergosterol as against the wild type. However, different results were obtained by Sud and Feingold [45] who have shown that the minimum inhibitory concentration for miconazole is 64-times higher in *S. cerevisiae* grown anaerobically in medium containing ergosterol, and the sporidia of an ergosterol-deficient mutant of *Ustilago maydis* with a metabolic block at C-14 demethylation is not inhibited by several C-14 demethylase inhibitors at concentrations that inhibit the wild-type strain [46].

Acknowledgements

We are grateful to Ms. Vivi Karathanansis for technical assistance. Funds were provided by the Ciba Geigy Corporation, the Herman Frasch Foundation and The Alabama Agricultural Experiment Station (Project No. ALA 5-877).

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