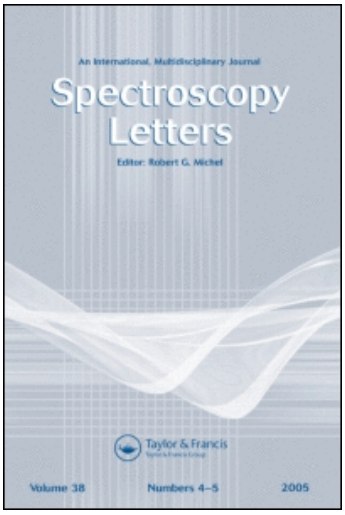
This article was downloaded by:
On: *30 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

A Spin Label Study of Membrane Alteration During Conversion of Fusarium sulphureum Macroconidia to Chlamydospores

R. W. Miller[a]
[a] Chemistry and Biology Research Institute, Ottawa

To cite this Article Miller, R. W.(1976) 'A Spin Label Study of Membrane Alteration During Conversion of Fusarium sulphureum Macroconidia to Chlamydospores', Spectroscopy Letters, 9: 12, 895 — 910
To link to this Article: DOI: 10.1080/00387017608067482
URL: http://dx.doi.org/10.1080/00387017608067482

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

SPECTROSCOPY LETTERS, 9(12), 895-910 (1976)

A SPIN LABEL STUDY OF MEMBRANE ALTERATION DURING CONVERSION OF FUSARIUM SULPHUREUM MACROCONIDIA TO CHLAMYDOSPORES

R.W. Miller

Chemistry and Biology Research Institute†
Research Branch, Agriculture Canada
Ottawa, Ontario. K1A 0C6

Downloaded At: 04:25 30 January 2011

Fusarium sulphureum is an important fungus belonging to a group of pathogenic Ascomycetes which cause root and vascular disease in cereal and vegetable crops. These organisms ordinarily grow as vegetative hyphae but under unfavorable conditions form macroconidia and chlamydospores as dispersal and survival propagules. The role of compositional and physical changes in cellular membranes during growth, survival and propagation of the pathogen provides the focus of this investigation.

A previous spin label study of the homeostatic control of membrane properties in Fusarium oxysporum over a wide range of growth temperature revealed compensating alterations in phospholipid and sterol composition of the isolated total membrane fraction (1). Membrane preparations from cells grown at 15°C contained more sterol (mainly ergosterol) than phospholipid yet exhibited roughly the same correlation time calculated for isotropic rotation of a fatty acid spin probe (16-doxyl stearate[1]) and a cholestane probe as did membranes isolated from 37°C grown cells (1). Membranes from

† Publication No. 924

Copyright © 1977 by Marcel Dekker, Inc. All Rights Reserved. Neither this work nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, microfilming, and recording, or by any information storage and retrieval system, without permission in writing from the publisher.

the latter cells had a phospholipid to total sterol ratio of approximately 1.5 on a molar basis. Fatty acid polyunsaturation varied in a reciprocal manner with growth temperature. Low temperature-grown cells produced membranes having a larger compliment of linolenic and linoelic acids than cells grown at 37°C. The high temperature limit for survival of F. oxysporum vegetative cells was found to be 39-40°C. This effect was associated with destructive alterations in membrane structure. Loss of membrane structure resulted in a change in the Arrhenius activation energies for motion of the fatty acid spin probe (1).

Morphological differences between hyphal cells, macroconidia and chlamydospores are well documented for Fusarium species (2,3). Function-related properties such as membrane fluidity and permeability have not been previously characterized for macroconidia or chlamydospores. In the present work the rate of incorporation and fate of fatty acid spin probes was determined in macroconidia and chlamydospores of F. oxysporum and F. sulphureum. During conversion of F. sulphureum macroconidia to chlamydospores, changes occurring in the distribution and metabolic degradation of the labels as well as overall changes in membrane lipid composition would be expected to be reflected in ESR spectra from labeled membranes of live cells.

Downloaded At: 04:25 30 January 2011

MATERIALS AND METHODS

Fatty acid spin labels were obtained from Syva Corp., Palo Alto, Ca. A stearamide[1] probe was prepared by the method of Hsia et al. (4) from precursors supplied by Aldrich Chem. Co. Biochemicals including mannitol were obtained from Sigma Chemical Co. Buffers, sodium dodecyl sulfate solutions and other chemicals were prepared with reagent grade materials from Fisher Scientific Co. Oleic acid was obtained from Nu Chek Prep.

[1] See footnotes

Inc., Elysian, Minn. Dodecyl guanidine acetate was a product of American Cyanamide Co.

Macroconidia containing 3 or 4 individual cells were prepared by inoculation of potato-sucrose agar plates with conidial suspensions (3). The cultures were grown at 25° under fluorescent lights for 7 days. Macroconidia were washed from the plates with sterile distilled water and sedimented in a clinical centrifuge. The cells were either used immediately in spin labeling experiments, stored at -20°C for lipid analyses, or converted to chlamydospores.

In order to measure the rate of uptake of 16-doxyl stearate, macroconidia were incubated either in a conversion medium (see below) or in a defined sucrose medium (6). In this experiment spin probe concentration was initially 40 μg/ml in the medium. After incubation for prescribed times at 25°C in a rotary shaking water bath (150 rpm, New Brunswick, Model G-76), cells were filtered rapidly on 1.2 μ Millipore filter and washed with a mixture of 0.002% ($\frac{W}{V}$) sodium dodecyl sulfate and 0.001 M potassium ferricyanide. This procedure both removed non-specifically adsorbed label (but not label bound inside the cells) and prevented internally bound label from becoming reduced by the actively respiring cells. The detergent had no effect on cell morphology as determined by transmission electron microscopy (or on ESR signals from macroconidia) at this concentration. Where indicated, sodium dodecyl sulfate was omitted from wash solutions.

Direct conversion of labeled macroconidia to chlamydospores was carried out in the dark at 37°C by the method of Barran *et al.* (5). Macroconidia ($5 \cdot 10^7$) were transferred into 100 ml of a mannitol-inorganic salts medium. The medium was not shaken. Conversion commenced within several hours and continued over an incubation period of 2-5 days at 37°C (5). After 5 days, eighty-five to ninety-five percent of the intact cells were present as

Downloaded At: 04:25 30 January 2011

fully developed conidial chlamydospores. These spores were harvested by centrifugation and sonicated for 2 min at 80% full power (about 300 watts) with a Biosonik III sonic oscillator having a 1.6 cm transducer probe. This treatment separated the individual chlamydospores which were then freed of debris by repeated washing with distilled water and centrifugation at 100 x g for 3 min in a clinical centrifuge. The dense chlamydospores sedimented more rapidly than debris or unconverted macroconidia which were removed from an unpacked layer above the chlamdospore pellet.

In separate experiments spin labels were included in the conversion medium. Under these conditions, cells were exposed to a constant external concentration (7.5 µg/ml) of label. This concentration of 16-doxyl stearate did not produce spin exchange broadening of signals in unconverted macroconidia during the entire incubation period. In a third type of experiment mature chlamydospores were exposed to spin labels dissolved in distilled water. Incorporation of 18 µg/ml oleic acid into the mannitol-inorganic salts conversion medium had no effect on efficiency of conversion. Washing labeled chlamydospores with detergent solutions altered ESR spectra as detailed below. The stearamide spin label (18 µg/ml) plus an equal amount of oleic acid replaced the fatty acid spin probe in some experiments.

ESR spectroscopy was performed with a Varian E-3 Spectrometer fitted with a Varian controlled temperature accessory. ESR signals from spin probes in intact cells were monitored in 0.8 mm inside-diameter pyrex capillaries having a 0.2 mm wall thickness.

All lipid isolations and quantitative analyses were carried out as previously described (1,7). Whole cells were extracted 3 times in boiling propanol by full speed agitation with a polytron disintegrator (Brinkman Instruments). Spores were counted with an A.O. Spencer hemocytometer after suitable dilution in distilled water. Constant dry weights of cells were

Downloaded At: 04:25 30 January 2011

obtained after drying aliquots of distilled water-suspensions at 80°C for 3 days.

RESULTS

Lipid Changes - Table 1 gives analyses of total phospholipid and sterol components extracted from macroconidia and chlamydospores. Compared with log phase hyphal cells grown at 25°C or 37°C (1), sterol to phospholipid ratios are increased, as expected, since the spores represent cells which are 5 to 10 days older than the 18 hr grown (log phase) hyphal cells. Sterols in a related species are known to increase in amount with age (8). Triglycerides were abundant in both macroconidia and chlamydospores to the extent of 10% or more of the dry weight of the cells. This neutral lipid is located in unordered, highly fluid lipid storage bodies (1,9) and would not be expected to be accessible to the respiratory chain or aqueous oxidants. Such bodies show no structure on transmission electron microscopy (2). Moreover, practically no molecular order was observed previously for spin labels dissolved in isolated triglyceride samples dried on glass plates (1).

Downloaded At: 04:25 30 January 2011

TABLE 1

Phospholipid and sterol content of macroconidia and chlamydospores of F. sulfureum

Lipid	Chlamydospores	Macroconidia
	mg per gm dry wt.	
Phospholipid	38	65
Free Sterol	37	30
Sterol Ester	6.1	10
Free Sterol / Phospholipid	0.97	0.46
Total Sterol / Phospholipid	1.10	0.62

Although a lower percentage of lipid was present in chlamydospores as compared with macroconidia on a dry weight basis, there appears to be some actual enhancement of the average phospholipid content of chlamydospores since it was determined that the latter weigh on the average four times as much as macroconidial cells.

Table 2 compares changes occurring in fatty acid composition of phospholipids and triglycerides on conversion of F. sulphureum macroconidia to chlamydospores. Since conversion requires elevation of the temperature to 37°C, a decrease in polyunsaturated fatty acids in lipids which are rapidly turning over would be expected as was demonstrated in log phase hyphal cells (1). Chlamydospore phospholipids exhibited a marked shift from linoleic acid to oleic acid as well as a 3 fold drop in polyunsaturated linolenic acid as compared to those in 25°C grown macroconidia. This, coupled with increases in sterol content, would be expected to increase viscosity in membrane lipid bilayers. Shifts in the unsaturation pattern for triglycerides were also observed for chlamydospores. The magnitude of this temperature-induced shift was less than that previously

Downloaded At: 04:25 30 January 2011

TABLE 2

Comparison of fatty acid composition of membrane and storage lipids of F. sulfureum macroconidia and chlamydospores

Lipid source	Fatty Acids 16:0	16:1	18:0	18:1	18:2	18:3	Δ/mol
			mol % [@]				
Chlamydospore phospholipids	26.5	0	3.4	41.8	23.5	5.0	1.04
Macroconidia phospholipids	21.4	0	2.5	4.9	51.7	19.2	1.66
Chlamydospore triglycerides	15.1	3.1	2.6	45.6	24.7	8.9	1.25
Macroconidia triglycerides	23.0	0.5	10.2	26.8	25.1	14.3	1.20

[@] Standard deviation for separate experiments was ± 3% of value given.

observed for hyphal triglycerides (1) and in fact little overall change in the number of double bonds per mol fatty acid (Δ/mol) was observed.

Incorporation of fatty acid spin labels - The ascending curve of Figure 1 shows the course of uptake of 16-doxyl stearate during incubation with the probe in a complete growth medium (6) with shaking at 25°C. Macroconidia did not germinate during this period. Another fatty acid probe, 12-doxyl stearate, gave a similar rate of uptake. The label rapidly accumulates up to 20 min to give a 6 fold concentration of label over the medium concentration based on double integration of the ESR signals. Thereafter, the amount of intracellular, bound label continues to increase more slowly giving a final concentration of over 10 fold compared with the ambient medium concentration. Figure 2, curve A, shows an

Downloaded At: 04:25 30 January 2011

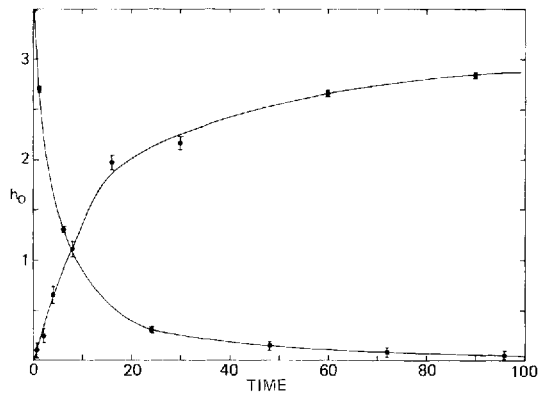


Fig. 1. Uptake and reduction of 16-doxyl stearate[1] by macroconidia. Axes for ascending curve represent time in minutes (abcissa) and height (h_0) of center resonance in arbitrary units multipled by 10^{-2} (ordinate) of ESR signal recorded at: receiver gain, $3.2 \cdot 10^5$; modulation amplitude, 0.8 gauss; center field strength, 3260 gauss. Macroconidia were incubated with the spin probe in a defined medium (5) with shaking at 25°C. Cells were quantitatively recovered, washed and introduced into capillary tubes as described under "Methods". Axes for descending curve represent time in hours (abcissa) and height (h_0) of center resonance in arbitrary units multiplied by 10^{-1} (ordinate) of ESR signal recorded at receiver gain, $5 \cdot 10^4$; modulation amplitude, 0.8 gauss; center field strength, 3260 gauss.

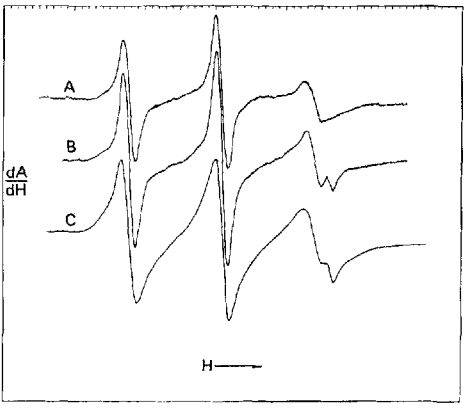


Fig. 2. ESR spectra of macroconidia labeled with 16-doxyl stearate. ESR spectra were recorded with the following instrumental parameters: microwave power, 25 milliwatts; modulation amplitude, 0.8 gauss; center field strength, 3260 gauss; field scan, ± 50 gauss; recorder chart speed 16 min for full scale scan; recorder time constant, 3.0 s. Curves represent spectra obtained at indicated times and spectrometer gains as follows: Curve A, 4 min, gain, $3.2 \cdot 10^5$; Curve B, 16 min, gain, $2.5 \cdot 10^5$; Curve C, 60 min, gain, $1.25 \cdot 10^5$.

Downloaded At: 04:25 30 January 2011

ESR spectrum of the probe in the washed cells after 4 min. The labeled fatty acid was tightly bound in a fluid matrix but displayed motion approaching isotropic rotation of the probe moiety. At 16 min (curve B) little change in the appearance of the spectum has occurred except that a small signal can be seen to be resolved from the main high field resonance. This signal arises from the fatty acid probe partitioning into the aqueous, intracellular fluid. This signal did not decrease in magnitude on further washing of the cells. After 60 min of labeling the signal from bound label exhibited some spin exchange broadening due to over-loading of the lipid environments of the label so as to allow spin-spin interaction between adjacent probe molecules (Curve C, Fig. 2). This phenomenon occurred only in macroconidia when probe concentration reached high levels near 300 µg (calculated from ESR signal) per ml of packed, wet cells. This was equivalent to 1 µg of probe per 63 µg of cell total lipid or 1 µg

probe per 15 µg total phospholipid. Calculated on a molar basis this would correspond to 1 mol probe per 4 mols phospholipid. For all experiments described below spin probe concentrations in spores were limited to a maximum of 1 probe molecule per 50 molecules of phospholipid fatty acid. In this way spin exchange broadening due to simple overloading of the cells with label was avoided.

The descending curve of Figure 1 shows the rate of reduction of 16-doxyl stearate in macroconidia (both Time and h_0 scales differ from those applicable to label uptake as detailed in the legend). The half time for label reduction was 4½ hours. The reduced label was reoxidizable with ferricyanide after this time. The initial magnitude of h_0 was slightly more than 1/5 that of the maximum signal of Fig. 2. Correlation time calculated for rotation of the probe (1,10) was 8.10^{-10} s. This value is very close to that previously reported for 25°C grown hyphae of F. oxysporum (1).

Figure 3 shows the kinetic course of changes in ESR signals from label bound in chlamydospores as a function of stage of development. At an incomplete stage in conversion of macroconidia (cf. Ref. 3, Fig. 3) three superimposed signals can be seen in curve A. At this stage the macroconidia are swollen but the chlamydospores are not completely formed as spherical cells with a thickened cell wall. Signal A_3 represents a small aqueous signal while signal A_2 represents label located in cell membranes and lipids. This signal corresponds to the probe in a "normal" hydrophobic environment causing slow tumbling motion of the label. Curve A_1 is a spin exchange broadened signal which does not arise from label evenly distributed at low concentration in membranes or lipids. As time progressed the chlamydospores became fully mature and signals A_3 and A_2 disappeared even though the external medium contained undegraded free spin label. After 5 days, only the broadened signal persisted (curve C). Signals corresponding to intracellular environments A_1 and A_2 (Fig. 3) were irreversibly lost

Downloaded At: 04:25 30 January 2011

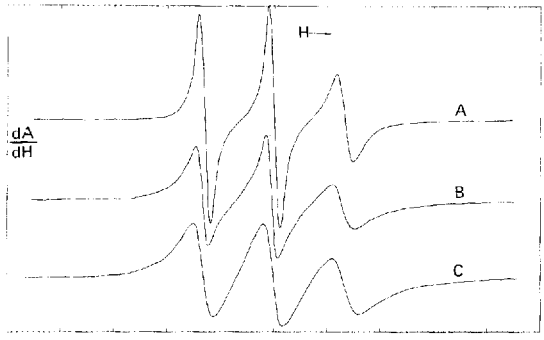


Fig. 3. Variation of chlamydospore ESR spectra with time. Macroconidia were introduced into conversion medium containing 7 µg 16-doxyl stearate per ml. Partially converted and fully mature chlamydospores were isolated by centrifugation and washed twice with 0.001 M potassium ferricyanide. Chlamydospores were concentrated as described under "Methods". ESR spectra were recorded at the indicated times at the specified receiver gain; Curve A, 48 hr, gain, $1.25 \cdot 10^5$; Curve B, 72 hr, gain, $8 \cdot 10^4$; Curve C, 132 hr, gain, $5 \cdot 10^4$. All spectra were obtained at: microwave power, 25 milliwatts; modulation amplitude, 1.0 gauss; recorder chart speed for full scale scan, 8 min; recorder time constant, 1.0 s.

Downloaded At: 04:25 30 January 2011

during this time and could not be restored with ferricyanide.

Figure 4 shows that the accumulation of the fatty acid spin probe at an unusually high concentration in a fluid environment is a property of fully developed chlamydospores. Curve A was obtained from F. oxysporum macroconidia after incubation in labeled conversion medium for 4 days at 37°C. These conidia did not convert to chlamydospores to an appreciable extent under the experimental conditions although they did contain many lipid bodies. Ten percent of these spores germinated while less than five percent converted to chlamydospores. The label signal showed no signs of spin exchange broadening and gave a rotational correlation time of $10 \cdot 10^{-10}$ s. Curve C was obtained from spin labeled F. sulphureum chlamydospores derived from macroconidia at 37°C. Although spores of both species were identically treated, only the F. sulphureum cells underwent conversion to chlamydospores and showed the spin exchange broadening phenomenon.

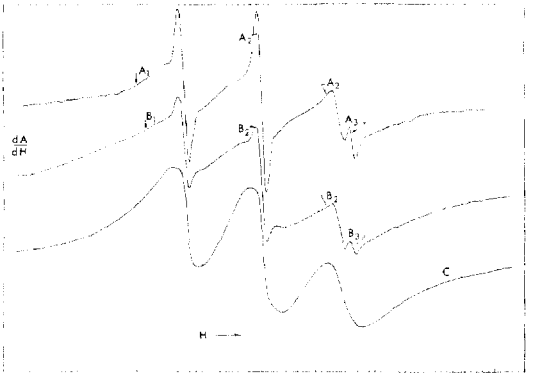


Fig. 4. ESR spectra of 16-doxyl stearate-labeled macroconidia and chlamydospores. ESR spectrometer settings were: receiver gain, $2.5 \cdot 10^4$; modulation amplitude, 0.8 gauss; field scan time, 16 min; recorder time constant, 1.0 sec. Curve A was obtained from macroconidia of F. oxysporum after 5 day incubation in conversion medium at 37°C. Curve B was obtained from 5 day F. sulphureum chlamydospores treated with 0.02% sodium dodecyl sulfate while Curve C was obtained with untreated chlamydospores.

Downloaded At: 04:25 30 January 2011

Addition of 16-doxyl stearate to unlabeled, mature chlamydospores gave labeled cells having ESR signals similar to curve C. The probe concentration in localized domains can be estimated to be about 10^{-3} M by comparison with spectra from isolated triglyceride solutions.

Treatment of the chlamydospores for 5 min at 50°C or with 0.02% dodecyl guanidine acetate gave the signal represented by curve B. These treatments redistributed the label and eliminated most of the exchange broadening effect. Treatment with 0.02% sodium dodecyl sulfate for 2 hrs or more also led to a complete redistribution of the label in the spores. Calculation of the correlation time after this or heat treatment (50°C) gave a value of $15 \cdot 10^{-10}$ s. Both of these treatments killed the chlamydospores. Incorporation of fatty acids such as oleic acid in the conversion medium eliminated the appearance of spin exchange broadened probe signals for both 16-doxyl stearate and the stearamide probe.

Incorporation of stearamide probe - Figure 5 shows the signals obtained with the stearamide probe located in membrane lipids of fully developed F. sulphureum chlamydospores. The spectrum of curve A was obtained at 23°C while curve B represents the 55°C spectrum. Correlation times calculated (11) for these spectra were $28 \cdot 10^{-10}$ s and $10 \cdot 10^{-10}$ s, respectively. Computer simulation of spectra for this label was carried out by the method of Polnaszek (12). Curves C and D represent close approximations of the stearamide probe spectra at 28°C and 55°C, respectively. Order parameters of 0.2 for the 23°C spectrum and 0.1 for the 55°C spectrum were used in determining the simulated spectra.

Downloaded At: 04:25 30 January 2011

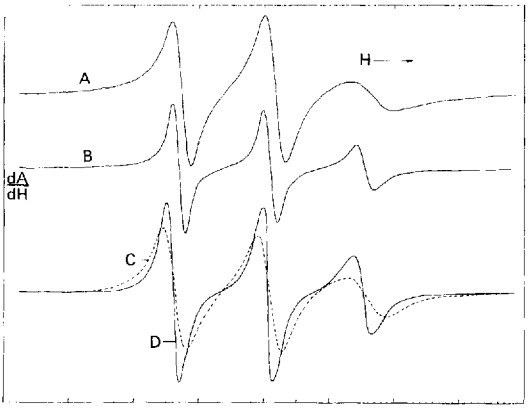


Fig. 5. Experimental and computer simulated spectra for stearamide probe in chlamydospores. Curves A and B were obtained with F. sulphureum chlamydospores 7 days after commencement of conversion in medium containing 18 µg/ml stearamide probe and 18 µg/ml oleic acid. ESR parameters were: receiver gain, Curve A, $6.2 \cdot 10^4$, Curve B, $4 \cdot 10^4$; microwave power, 25 milliwatts; modulation amplitude, 0.8 gauss; field sweep; centre field value and recorder parameters as in Fig. 1. Temperature for curve A was 23°C, curve B, 55°C. Curves C and D are computer simulated spectra obtained by the method of Polnaszek (12). Parameters used in simulation were: g values, $g_x = 2.0061$, $g_y = 2.0027$, $g_z = 2.0087$; hyperfine splitting constants, $A_x = 5.5$ gauss, $A_y = 31.2$ gauss, $A_z = 6.5$ gauss; correlation time, curve A, $28 \cdot 10^{-10}$ s, curve B, $10 \cdot 10^{-10}$ s; order parameter, curve A, 0.18, curve B, 0.10.

The stearamide probe was more rapidly reduced by respiratory activity above 25°C than was the fatty acid probe. Removal of the labeled chlamydospores from the stearamide-containing conversion medium led to a rapid loss of the label signal ($T_{\frac{1}{2}} < 1$ hr). No spin exchange broadened component was observed with this probe. However, the solid, insoluble properties of this label necessitated the inclusion of oleic acid in the incubation medium for incorporation of the label into cellular lipids. Under these conditions neither the stearamide probe nor 16-doxyl stearate were concentrated in a cellular domain which caused spin exchange broadening.

DISCUSSION

Changes in polyunsaturated fatty acids associated with conversion of F. sulphureum macroconidia to chlamydospores follow a pattern similar to those reported (1) for log phase hyphae of F. oxysporum when grown at 37°C. Since the conversion requires incubation of macroconidia at 37°C for several days, it can be assumed that fatty acid turnover and resynthesis of phospholipids de novo is responsible for the decrease in unsaturation which accompanies the conversion process.

Since sterols, mainly ergosterol (1), accumulate in stationary phase hyphal cells (9) as well as in macroconidia and chlamydospores, the observed changes in sterol content cannot be associated uniquely with sporulation. High triglyceride levels in spores and stationary phase hyphal cells presumably provide storage metabolites for regrowth under favorable conditions.

Although, chlamydospores showed somewhat more restricted motion for 16-doxyl stearate after intracellular distribution in the presence of 0.02% w/v sodium dodecyl sulfate (50% increase in rotational correlation time), this effect could be due to modification of membrane properties by the detergent. Under the conditions which were employed, this detergent did not lyse the chlamydospores as determined by permanganate fixation and electron microscopy.

Downloaded At: 04:25 30 January 2011

Macroconidia of F. oxysporum which were subjected to the same temperature regime as macroconidia of F. sulphureum did not convert to chlamydospores but remained viable. Some of these cells germinated and all underwent the same alterations in lipid composition. However, no decrease in fluidity or other changes in the hydrophobic mileau of 16-doxyl stearate was detected by ESR spectroscopy.

One major effect of conversion to chlamydospores which occurred in the behavior of membranes and lipids labeled with 16-doxyl stearate was the irreversible loss of the signal from fluid regions of membranes and lipids through reduction and further metabolism of the probe. Although the label remained in the conversion medium, it was no longer distributed throughout the membranes. Instead, the spin label became highly localized in some domain where the unpaired electrons experienced spin-spin coupling interactions as evidenced by the spin exchange broadened ESR signals (Fig. 3 and 4). The exact location of this label in the cell is unknown but it cannot be removed by aqueous detergent washing. It is conceivable that the label could be associated with the cell wall which increases in mass during conversion. No spin label associates with cell wall in hyphal or macroconidial cells, however (1). If the fatty acid probe were bound to solid phase materials such as the cell wall polysaccharides or proteins, a spectrum showing immobilization of the probe rather than a spin exchange broadened fluid environment would result. This type of powder spectrum was never observed for chlamydospores.

It is clear that F. sulphureum chlamydospores are unable to take up 16-doxyl stearate and distribute it in the membrane bilayers as hyphal, microconidial and macroconidial cells do. Such distribution in chlamydospores requires the addition of detergents or heating above lethal temperatures ($50^{o}C$). Oleic acid acted as a non-lethal permeant and allowed the stearamide probe to distribute within the membrane lipids. Two findings substantiate the view that this probe locates mainly in membranes rather than in trig-

Downloaded At: 04:25 30 January 2011

lyceride bodies. The probe exhibits molecular motion characteristic of slow tumbling and is located in an environment having an order parameter (13) greater than 0.2 as determined by matching computer simulated spectra (12) with the observed spectra. No value of correlation time combined with a lower order parameter produced calculated spectra having the required intensity ratio of the 3 nitroxide resonances. Triglycerides which are known to have virtually no ordered structure either in vitro or in vivo, cannot provide such an environment for this probe. Since the stearamide probe unpaired spin would be expected to locate in the polar region of membrane lipid bilayers, it would be expected to be rapidly and completely reduced by respiratory activity of membranes in equilbrium with the mitochondria. This label was reduced more rapidly than 16-doxyl stearate, and had a half life of less than 1 hour at 25°C in macrocondial membranes. The necessity for adding oleic acid for incorporation of the stearamide label into fungal membranes points to the requirement for partition of lipids between external fluid environments (lipid or aqueous) and cellular lipid environments for efficient uptake of the lipid.

The finding that macroconidia reduce and metabolyze doxyl-stearate spin probes during conversion to chlamydospores, and that the latter, when mature, do not readily distribute these probes in membrane lipids, points to an important alteration in the permeability of these spores to amphipathic metabolites.

Downloaded At: 04:25 30 January 2011

ACKNOWLEDGEMENTS

The author thanks Wyman C. Adams for excellent and consistant technical assistance.

Footnotes: 1. 16-doxyl stearate, designation for the N-oxyl-4'-4' -dimethyloxazolidine derivative of 16-keto-stearic acid. Stearamide probe; designation for 4-stearamide-1-oxyl-2,2,6,6-tetramethyl piperidine.

REFERENCES

1. R.W. Miller, and I.A. de la Roche. Biochim. Biophys. Acta 443:64 (1976).

2. I.L. Stevenson, and S.A.W.E. Becker. Can. J. Microbiol. 18:997 (1972).

3. E.F. Schneider, and W.L. Seaman. Can. J. Microbiol. 20:247 (1974).

4. J.C. Hsia, L.H. Piette, and R.W. Noyes. J. Reprod. Fert. 20:147 (1969).

5. L.R. Barran, E.F. Schneider, and W.L. Seaman. Can. J. Microbiol. In press. (1976).

6. R.W. Miller. Arch. Biochem. Biophys. 146:256 (1971).

7. L.R. Barran, R.W. Miller, and I.A. de la Roche. Can. J. Biochem. 22:557 (1976).

8. C. Madhosingh. Phytopath. In press. (1976).

9. J.D. Weete. Fungal Lipid Biochemistry, Plenum Press, New York, p. 30. (1974).

10. B. Cannon, C.F. Polnaszek, K.W. Butler, L.E.G. Eriksson, and I.C.P. Smith. Archives Biochem. Biophys. 167:505 (1975).

11. S.P. Verma, L.J. Berliner, and I.C.P. Smith. Biochem. Res. commun. 55:704 (1973).

12. C.F. Polnaszek. Unpublished. (1976).

13. W.L. Hubbell, and H.M. McConnell. J. Amer. Chem. Soc. 93:314 (1971).

Received: 10-20-76
Accepted: 11-8-76

Downloaded At: 04:25 30 January 2011