DERMATOPHYTE LIPIDS – COMPOSITION AND REGULATION OF PHOSPHOLIPIDS.

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ABSTRACT

This review presents the extensive work carried out on lipid components of dermatophytes, their biosynthesis, turnover and regulation. It emerges from the work done so far that the pathways of lipid biosynthesis/ degradation and the lipid composition in dermatophytes are similar to those in yeasts and other fungi. Second messengers (Ca^{2+} , cAMP) were demonstrated to have a regulatory role in phospholipid metabolism and they mainly act by stimulating Ca^{2+} /CaM or cAMP dependent protein kinase(s). Both these kinases were purified and characterized in *Microsporum gypseum*. Further work is being carried out to elucidate the molecular mechanism of regulation of phospholipid metabolism by these second messengers.

KEY WORDS: Dermatophytes, lipids, phospholipids, second messengers, regulation, protein kinases.

INTRODUCTION

Dermatophytes are a group of pathogenic fungi that cause infection of the superficial keratinized tissues such as epidermis, hair and nails. Lipids of dermatophytes have received considerable attention during the past few decades, as they are the prime targets of antifungal drugs. Several reports are available showing the importance of fatty acids, sterols and other lipid components for growth, sporulation and germination, which clearly indicate the involvement of lipids in various physiological processes. Moreover, the possible involvement of fungal lipids in virulence, allergic reactions, pathogenicity and drug sensitivity have been reported (1-4). Keeping in view the importance of lipids in dermatophytes, this article reviews the different aspects of lipid metabolism with special reference to the role of intracellular second messengers (Ca^{2+} & cAMP) in

the regulation of phospholipid biosynthesis in dermatophytes.

LIPID COMPOSITION

Lipids are the prime carbon source and substrate for polysaccharide synthesis in dermatophytes and act as energy stores (2). Lipids of 10 species of dermatophytes were reported. Most dermatophytes contain total lipids in the range of 7-19.8% of dry wt (5,6). The cell wall lipids which impart rigidity and protection to the cell were found to range between 3.1-6.6% of dry wt in these dermatophytes (7,8). The total lipids of *Microsporum*

Abbreviations: Ca^{++} , Calcium; cAMP, cyclic adenosine monophosphate; CAM, Calmodulin; TPL, Total phospholipids; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, Phosphatidylserine; PG, Phosphatidylglycerol; LPC, Lysophosphatidylcholine; CDP, Cytidine diphosphate; PI, Phosphatidylinositol; CL, Cardiolipin; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; IP3, inositoltriphosphate, TFP, Trifluoperazine; TG, triacylglycerols; U, Unsaturated fatty acids; S, Saturated fatty acids.

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gypseum and *Epidermophyton floccosum* were observed to be significantly lower as compared to those of *Trichophyton mentagrophytes* at all phases of growth (9,10). Triglycerides are the major neutral lipid component whereas monoglycerides (MG), diglycerides (DG), free fatty acids, sterol and sterol esters are the minor ones (11). These fungi contain a homologous series of saturated and unsaturated fatty acids ranging from C₆ to C₂₂. The short chain fatty acids were detected in significant amount only during the early growth phase (12). Triglycerides which constitute the major fraction of neutral lipids in dermatophytes (13) have been regarded as an energy reserve during unfavourable conditions and also help to keep fatty acids under non toxic limits and stimulate reproduction (14), however, no specific function was assigned to MG and DG. Ergosterol was shown to be the major component among sterols in *Trichophyton rubrum* as it constituted 71% of the total free sterol of this fungus (15). Other sterols like ergocalciferol, brassicasterol and campesterol are present in small amounts.

Phospholipid composition of various species of dermatophytes were found to be different but in most of the dermatophytes the major phospholipids are PC, PE, PS, PI, PG, LPC and some unusual lipids (4,5,13-16). Table 1 depicts the comparative account of phospholipids present in *Epidermophyton floccosum*, *Microsporum gypseum*, *Microsporum cookei* and *Trichophyton rubrum*. The phospholipid composition of *Microsporum gypseum* is similar to that of *Epidermophyton floccosum* except for the complete absence of cardiolipin in the former (17).

Besides phospholipids, an unusual lipid containing amino acid was detected in the polar lipid fraction of *Microsporum gypseum* and *Epidermophyton floccosum* (17). However, the unusual lipid of *Microsporum gypseum* was found to be structurally different from *Epidermophyton floccosum*. Since lipids are charged molecules, these unusual lipid components probably substitute for some phospholipids that are absent in these organisms (16).

Table 1 - Total phospholipid content of various species of dermatophytes.

Organism	Culture	Total phospholipid content (mg/g dry wt.)
<i>Microsporum gypseum</i>	Stationary	11.1
<i>Microsporum gypseum</i>	Shake	16.1
<i>Epidermophyton floccosum</i>	Stationary	8.2
<i>Epidermophyton floccosum</i>	Shake	13.9
<i>Microsporum cookei</i>	Stationary	14.3
<i>Trichophyton mentagrophytes</i>	Stationary	35.9

Data from Chopra and Khuller (2)

Biosynthesis and turnover of phospholipids

Turnover of lipids as studied by pulse chase experiments using radioactive precursors suggest that among phospholipids, PC exhibits maximum rate of synthesis and degradation followed by PE and PS (17-20). These studies also reveal the existence of two phospholipid pools with different turnover rates in fungi. The pool with fast turnover was proposed to be located in metabolically active regions of the organism as compared to the pool with slow turnover (17).

In vivo incorporation studies with nitrogenous bases revealed that PE is mainly synthesized by PS decarboxylation and methylation pathways (18). The results of [¹⁴C]-methionine and [¹⁴C]-choline incorporation studies also indicated the presence of two pools of lipids containing choline in *Microsporum gypseum* similar to *Epidermophyton floccosum* (19,21). Studies also demonstrated the existence of a common pathway for neutral lipid as well as phospholipid synthesis in *Microsporum gypseum*.

Biosynthetic enzymes of phospholipids

Information available regarding the enzymes involved in biosynthesis of phospholipids in dermatophytes is limited. Different pathways exist

for the biosynthesis of phospholipids as depicted below (Fig. 1) but all the pathways share a common intermediate – phosphatidic acid (PA).

Enzymes involved in the biosynthesis of PA viz. glycerol kinase and acyltransferase have been well-characterized in dermatophytes (22,23). Biosynthesis of phospholipids takes place primarily by two routes i.e. CDP-base and CDP-diacylglycerol pathways. PC and PE are synthesised by CDP-base pathway while CDP-diacylglycerol pathway is operative for the synthesis of PS, PI and CL. PLs can also be synthesized by an alternative pathway like methylation, base exchange and acylation-deacylation reactions (23-25). Initially base exchange and PS-decarboxylase were thought to be responsible for PE synthesis in *Microsporium gypseum* (20) but later on CDP-base pathway was established for its synthesis. However, the relative contribution of each pathway to cellular PE is not known. Some of the key enzymes of these pathways have been identified and well characterized in dermatophytes (Fig. 1). The synthesis of PI in mammals and yeasts (26) was

shown to occur through the CDP-diacylglycerol pathway similar to PS. White and Hawthorne (27) demonstrated that PI could also be synthesized by some other pathway in yeast which does not involve the presence of CDP-diacylglycerol.

Catabolism of lipids

A great deal of interest has been generated in the degradative enzymes of phospholipids in dermatophytes because of their possible involvement in initial stages of infection. Catabolism of phospholipids in dermatophytes has been found to be regulated by enzymes, presented in Fig. 2.

Higher incorporation of [^{14}C]-acetate into TG other than neutral lipids suggests the presence of an active phosphatidate phosphatase in *Microsporum gypseum* and *Epidermophyton floccosum* (24,28,29). Phospholipase A has been detected in *Trichophyton rubrum*, *Epidermophyton floccosum*, *Microsporum cookei*, and *Trichophyton mentagrophytes* (23,30,31). The phospholipase activity is either of A type (*Epidermophyton floccosum*), A_2 type (*Microsporum*

Figure 1. Biosynthetic pathways of major phospholipids in *M. gypseum*. Thick lines represent enzymes identified.

Figure 2. Tentative pathways of phospholipid catabolism in dermatophytes.

cookei), or both A and A₂ type (*Trichophyton mentagrophytes*) (30). Lysophospholipase helps in the removal of cytotoxic lysophospholipids produced by the action of an intracellular or extracellular phospholipase A in these dermatophytes (31). In addition to these enzymes, phospholipase C and glycerophosphocholine diesterase activity was also detected in these fungi (30, 31). Detection of intracellular lipase in *Epidermophyton floccosum* (32) showed that this enzyme participates in cellular lipid metabolism of the fungus besides assisting in possible invasion of host tissues as described earlier (32-34).

Role of second messengers in the regulation of phospholipid metabolism

There are two major signaling pathways that control cellular functions in most of the systems. One is kinase pathway coupled to cAMP; while the second is the Ca²⁺ messenger system in which the

receptor activated phospholipase C hydrolyzes phosphatidylinositol-4,5- bisphosphate. Both the pathways are interrelated and act synergistically to regulate cellular functions (35). Both cAMP and calcium have been shown to regulate phospholipid synthesis in *Microsporum gypseum*, a dermatophyte (36,37).

Regulation of phospholipids by cAMP

The information about the regulation of phospholipid synthesis by cAMP is available mainly in mammals (38,39), however, in fungi, no molecular signal that has a direct role in regulating cAMP levels has been identified. The functions of cAMP are determined by its endogenous level which in turn depends upon its relative rate of synthesis and degradation by diverse families of adenylate cyclase(s) and phosphodiesterase(s) (40).

Since cAMP does not cross the plasma membrane, the manipulation in intracellular levels of cAMP is done with various agents (atropine, aminophylline, cholera toxin, forskolin, dibutyryl cAMP etc.) as shown above. Evidence for the involvement of cAMP in phospholipid synthesis in dermatophytes has come from the studies where the exogenous supplementation of dibutyryl cAMP enhanced the total lipids, phospholipids and individual lipid fractions (41). Maximum increase was found in the PC biosynthesis, thus suggesting that cAMP regulates phospholipid metabolism mainly through its action on PC biosynthetic enzymes. Also the increased content of phospholipids in aminophylline grown cells was correlated to the stimulation in activities of various biosynthetic enzymes (36).

The correlation between decreased cAMP levels and lipid synthesis was also seen by using

the cAMP modulator, atropine which decreased cAMP levels by 2.3 fold. A decreased activity of both glycerol kinase and acyltransferase was observed in the cells (Table 2) grown in the presence of this modulator (42).

cAMP induced maximum alterations in the choline containing lipids of aminophylline and atropine grown cells (43). The regulation of both CDP choline and methylation pathways for PC biosynthesis using specific radiolabelled precursors revealed significant changes in the incorporation of [^{14}C]-choline in aminophylline/atropine grown cells respectively, indicating that CDP-choline pathway is probably regulated by cAMP involving choline kinase and cytidyltransferase. However, methylation pathway was found to be more influenced by cAMP than CDP-choline pathway (43).

Table 2 - Activities of enzymes of phospholipid biosynthesis in *Microsporium gypseum* in presence of cAMP modulators.

Enzyme	Control	Aminophylline grown cells (4 mM)	Atropine grown cells (2mM)
Glycerol kinase	10.4 $\pm$ 1.3	19.0 $\pm$ 0.9	5.6 $\pm$ 0.9**
Glycerol 3-(P) acyltransferase	17.7 $\pm$ 0.1	34.4 $\pm$ 0.7**	14.4 $\pm$ 1.2*
Choline kinase	0.5 $\pm$ 0.1	0.8 $\pm$ 0.0**	0.5 $\pm$ 0.1 ^{NS}
Cytidyltransferase	41.5 $\pm$ 0.2	55.5 $\pm$ 4.6*	19.1 $\pm$ 3.4**

The enzyme activities are expressed as nmoles/mg protein/h

*p<0.05, **p<0.01 [Data from Bindra A and Khuller GK, (42)].

NS - Nonsignificant

Regulation of phospholipids by calcium/calmodulin

Increased levels of Ca^{2+} leads to the activation of various physiological processes such as gluconeogenesis, glycogenolysis, steroidogenesis and cell proliferation (44,45). It has been shown to regulate the synthesis of macromolecules like DNA, RNA and protein during dimorphic transition in fungi (46), the release of lipase in *Fusarium oxysporum* (47). The intracellular effects of Ca^{2+} are mediated by a family of Ca^{2+} binding protein, of which CaM is the most widely distributed and versatile (48). However, the role of Ca^{2+} and CaM in lipid biosynthesis has been investigated to a limited extent. In dermatophytes, the regulation of phospholipids by calcium in *Microsporum gypseum* have been studied by supplementing calcium in the growth medium. Calcium grown cells exhibited a significant increase (1.6 fold) in total as well as individual phospholipids which was further supported by increased incorporation of labelled acetate/phosphate into total phospholipids and increased activity of key phospholipid biosynthetic enzymes (glycerol kinase and glycerol-3-PO acyltransferase) in calcium grown cells (42,44). An increase in fatty acid synthesis was observed in calcium grown cells, which suggests an increase in phospholipids due to increased synthesis of fatty acids, which ultimately enhanced the activity of glycerol-3-PO₄ acyltransferase (37).

In *Microsporum gypseum*, a dermatophyte, calmodulin-like protein has been identified and purified but no specific function was assigned to it (50). The effect of calcium on phospholipid synthesis is possibly mediated by calmodulin as suggested in earlier studies (48). Studies using antagonists of calmodulin viz. phenothiazine and trifluoperazine which inhibit calmodulin through non stereospecific hydrophobic interactions, strongly suggest that calmodulin regulates phospholipid synthesis in *Microsporum gypseum* at the level of fatty acid synthesis (53).

Mechanism of action of second messengers

Second messengers, cAMP and calcium exert their effects through cAMP and Ca^{2+} /CaM

dependent protein kinases respectively, which phosphorylate a number of endogenous proteins. These kinases have been isolated from yeast, algae and fungi and are functionally identical to those of mammalian cells. Phosphatidylserine-synthase and cytidylyl transferases have been shown to be regulated by phosphorylation by cAMP-dependent protein kinase(s) in yeast (51,52).

In *Microsporum gypseum*, the presence of protein kinase activity has been reported and this activity was observed to be significantly increased in aminophylline and calcium grown cells, indicating that the modulators which enhance phospholipid synthesis in dermatophytes also activate the protein kinase activity (54).

A Ca^{2+} /CaM dependent-protein kinase (CaMPK) dependent protein kinase (PKA) have been purified to homogeneity from soluble extract of *Microsporum gypseum* (55,56). Addition of KN-62 (a specific inhibitor of Ca^{2+} /calmodulin dependent protein kinases) and polyclonal antibodies (raised against purified CaMPK of *Microsporum gypseum*) to the cell extract, leads to the inhibition in the incorporation of labelled acetate into total phospholipids in this fungus (55). This suggests a possible involvement of Ca^{2+} /calmodulin via Ca^{2+} /calmodulin dependent phosphorylation in phospholipid synthesis in *Microsporum gypseum*. However, more work is required to elucidate the exact mechanism of the regulation of phospholipid synthesis in *Microsporum gypseum*. Further work is focussed on the identification of physiological substrates of these protein kinases, which have a regulatory role in phospholipid metabolism.

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