J. Phytol. Res. 9 (1): 1-6, 1996

LIPID METABOLISM IN MEMBRANES OF *NEOVOSSIA INDICA*, KARNAL BUNT OF WHEAT UNDER MAGNESIUM AND CARBON STARVATION

SUSHMA PHUTELA and J. S. ARNEJA

Department of Biochemistry, Punjab Agricultural University, Ludhiana-141 004, India.

Starvation of *Neovossia indica*, a causative organism of smut disease of wheat by magnesium and carbon revealed that dry matter, lipids and protein synthesis in membranes were slowed down considerably. Under these conditions, phosphatidyl choline among phospholipids and free fatty acid among neutral lipids were adversely affected and appeared to be partially replaced by triglycerides, sterols and sterol esters. The relative percentage of phosphatidic acid and phosphatidyl inositol in the membrane increased while that of phosphatidyl choline decreased with the increase in the period of starvation. Polyglycerophosphatides and cardiolipins have been assigned the role of temporary reservoir of phosphatidic acid residue under starvation conditions. Among these two, carbon starvation had more adverse effect on synthesis of membrane constituents. These effects were more pronounced towards longer periods of starvation i.e., 16 days. Furthermore, the adverse effects of starvation were reversed by restoring the deficient nutrients.

Keywords: Fungal lipids; Membrane lipids; Mycelial lipids; *Neovossia indica*.

Introduction

Karnal bunt Neovossia *indica* is one of the most considerable concern among the smut diseases of wheat, as till the present time, there are no true resistant varieties against this organism. There is a great need to understand the properties of this pathogen belonging to fungal class Basidiomycetes. Few aspects of lipid metabolism in this organism have been reported[1] but the information on the membrane lipids and proteins seems to be negligible. As biological membranes are the sites of a large variety of cellular processes ranging from permeability, transport, excitability to intercellular interactions, morphological differentiation and diffusion, thus studies on structural relationship between membrane components become necessary in order to understand their functional properties. The present work was, therefore, undertaken to study the biosynthesis of membrane structural components in *Neovossia indica* in relation to membrane integrity under stresses of essential growth nutrients i.e., magnesium and carbon.

Materials and Methods

The cultures of *N. indica* were maintained by quarterly transfer on the agar slants using potato dextrose agar medium. For magnesium and carbon stravations, fungus was initially grown in basal synthetic medium of Chahal and Gray[2] and 8 days old cultures were transferred to medium deficient in magnesium and carbon and starved for 4, 8, 12 and 16 days. Fungal mats were retransferred after 8 days of interval to subsequent medium deficient in magnesium and carbon. Effect of restoration of these elements was studied by transferring the starved mycelium to the medium having different levels of magnesium and carbon replenished. Membranes were isolated from fungal mats by differential centrifugation technique[3]. Total lipids were extracted by the method of Folch *et al.*[4] Techniques of TLC was employed for the fractionation of different lipid classes. Total sterols were determined by the method of Stadtman[5] and free fatty acids by the method of Lowry and Tinsley[6]. The determination of total phospholipids was carried out by the

method of Ames[7]. Preparative TLC was employed for the separation of different classes of phospholipids.

Results and Discussion

The synthesis of both the membrane lipids and proteins were adversely affected by starving the organism of magnesium and carbon supply (Table 1 and 2). Among these two, carbon starvation had a more deleterious effect on accumulation of membrane dry matter, lipids and protein than that of magnesium. It is evident that at 16 days of starvation, lipids and protein were reduced by more than 50 percent under carbon starvation and more than 30 percent under magnesium starvation as compared to that under the normal supply of the growth nutrients. The starvation effects in both the cases got more pronounced as the period of starvation increased from 12 to 16 days. Restoration of the deficient nutrients to organism resulted in regaining the anabolic processes towards normal, which is evident from the increased synthesis of membrane and its constituents which suffered from the starvation effect.

Among the different lipid fractions, the accumulation of phospholipids and free fatty acids (FFA) declined continuously whereas that of triglycerides (TG), sterol esters and hydrocarbons (SEH) increased with the increase in the period of starvation of both magnesium and carbon (Table 1 and 2). The relative percentage of sterols was increased under magnesium starvation, while decreased under carbon starvation. Thus, under starvation conditions phospholipids in the membrane appeared to be partially replaced by sterols, triglycerides and sterol esters without any adverse effect on membrane viability. An increased level of sterols and sterol/phospholipid ratio had also been reported in the membranes of *Saccharomyces cerevisiae* under starvation of essential growth nutrients[8]. The decrease in relative percentage of FFA may be due to their rapid utillization or slower rate of synthesis.

Analysis of individual phospholipid classes revealed that phosphatidic acid (PA), phosphatidyl inositol (PI), polyglycero-phosphatide (PGP) and cardiolipins (CL) progressively accumulated at higher rate while phosphatidyl choline (PC) declined as the period of starvation was increased to 16 days both under magnesium and carbon starvation (Table 3 and 4). However, the relative percentage of phosphatidyl ethanol amine (PE) declined only under magnesium starvation (Table 3). Although, Gibson *et al.*[9] reported the requirement of magnesium ions for methyl transferases involved in conversion of PE to PC, yet our results indicated the involvement of magnesium not only for the transmethylation of PE but also for its own synthesis, as is evident from the observed decrease in level of both PE and PC under magnesium starvation. The observed decline in accumulation of PC under carbon starvation is explained on the basis of slower growth rate and hence lesser synthesis of membrane and its constituents. Since biosynthetic activities cannot be sustained under depleted carbon supply, the synthesis of major phosphatide is reduced.

The increase in level of PA, PGP and CL is observed under unfavorable conditions support the hypothesis partially enunciated earlier from these laboratories [10,11]. It is hypothesized that under adverse conditions of growth where biosynthesis of PC, the major phosphatide is curtailed, the available PA in the cell is stored in the temporary pool of PGP and CL, which can supply the PA residues

Table 1. Effect of magnesium stravation and replenishment on the membrane and its constituents.

Starvation pattern	Membrane DM	Total lipids	Phospho-lipids	Sterols	FFA	TG+SEH*	Proteins
	mg dl^{-1}	%	%	%	%	%	%
N → N → N → N (8) (8) (8) (8)	68.0	26.5	22.8	8.6	24.0	44.5	51.0
N → S (8) (4)	66.0	25.2	20.7	9.0	20.2	51.9	43.0
N → S (8) (8)	59.6	23.8	19.0	10.1	19.1	56.3	42.0
N → S → S (8) (8) (8)	54.6	21.2	17.8	12.2	18.2	62.3	37.0
N → S → S (8) (8) (8)	51.6	17.2	16.4	13.0	16.0	72.5	34.0
N → S → S → N/2 (8) (8) (8) (8)	61.0	18.0	17.7	10.2	18.0	70.1	39.0
N → S → S → N (8) (8) (8) (8)	61.2	22.0	19.9	8.0	21.9	61.5	42.0

N = Normal medium, S = Magnesium starved medium, N/2 = Magnesium replenished half the normal. Figures in parentheses indicate the days of incubation. *TG+SEH(%) = 100-[Phospholipids (%) + Sterol (%) + FFA(%)].

Table 2. Effect of carbon starvation and replenishment on the membrane and its constituents.

Starvation pattern	Membrane DM	Total lipids	Phospho-lipids	Sterols	FFA	TG+SEH*	Proteins
	mg dl^{-1}	%	%	%	%	%	%
N → N → N → N (8) (8) (8) (8)	34.0	26.5	22.8	8.6	24.0	44.5	51.0
N → S (8) (4)	31.7	20.0	22.3	8.3	17.5	51.9	33.0
N → S (8) (8)	25.7	17.2	21.0	7.4	15.1	56.3	30.0
N → S → S (8) (8) (4)	21.1	15.0	17.3	6.2	14.1	62.3	26.0
N → S → S (8) (8) (8)	10.9	11.5	13.2	2.1	12.2	72.5	22.0
N → S → S → N/2 (8) (8) (8) (8)	15.2	13.2	19.1	4.2	12.5	70.1	23.0
N → S → S → N (8) (8) (8) (8)	22.9	15.0	21.3	4.2	13.0	61.5	29.0

N = Normal medium, S = Carbon starved medium, N/2 = Carbon replenished half the normal. Figures in parentheses indicate the days of incubation. *TG+SEH(%) = 100-[Phospholipids (%) + Sterol (%) + FFA(%)].

Table 3. Effect of magnesium starvation and replenishment on different phosphatides in membranes.

Starvation pattern	Phosphatide (Relative percentage)				
	PA	PI	PC	PE	PGP+CL
N → N → N → N (8) (8) (8) (8)	5.0	7.3	66.7	11.7	9.2
N → S (8) (4)	6.9	8.2	63.7	11.0	10.1
N → S (8) (8)	8.5	10.6	55.4	10.3	15.1
N → S → S (8) (8) (4)	10.2	11.5	53.6	7.3	17.4
N → S → S (8) (8) (8)	14.1	12.3	46.2	6.0	21.4
N → S → S → N/2 (8) (8) (8) (8)	12.3	9.7	53.2	8.6	16.2
N → S → S → N (8) (8) (8) (8)	6.6	8.2	62.7	10.7	11.7

N = Normal medium, S = Magnesium starved medium, N/2 = Magnesium replenished half the normal. Figures in parentheses indicate the days of incubation.

Table 4. Effect of carbon starvation and replenishment on different phosphatides in membranes.

Starvation pattern	Phosphatide (Relative percentage)				
	PA	PI	PC	PE	PGP+CL
N → N → N → N (8) (8) (8) (8)	5.0	7.3	66.7	11.7	9.2
N → S (8) (4)	6.5	8.4	60.5	12.5	12.1
N → S (8) (8)	8.2	9.5	55.4	13.9	13.1
N → S →S (8) (8) (4)	10.1	10.9	51.4	13.9	13.7
N → S → S (8) (8) (8)	12.0	12.3	46.2	14.9	14.6
N → S → S → N/2 (8) (8) (8) (8)	9.3	10.9	54.1	13.6	12.1
N → S → S → N (8) (8) (8) (8)	8.3	9.0	59.4	13.6	10.2

N = Normal medium, S = Carbon starved medium, N/2 = Carbon replenished half the normal. Figures in parentheses indicate the days of incubation.

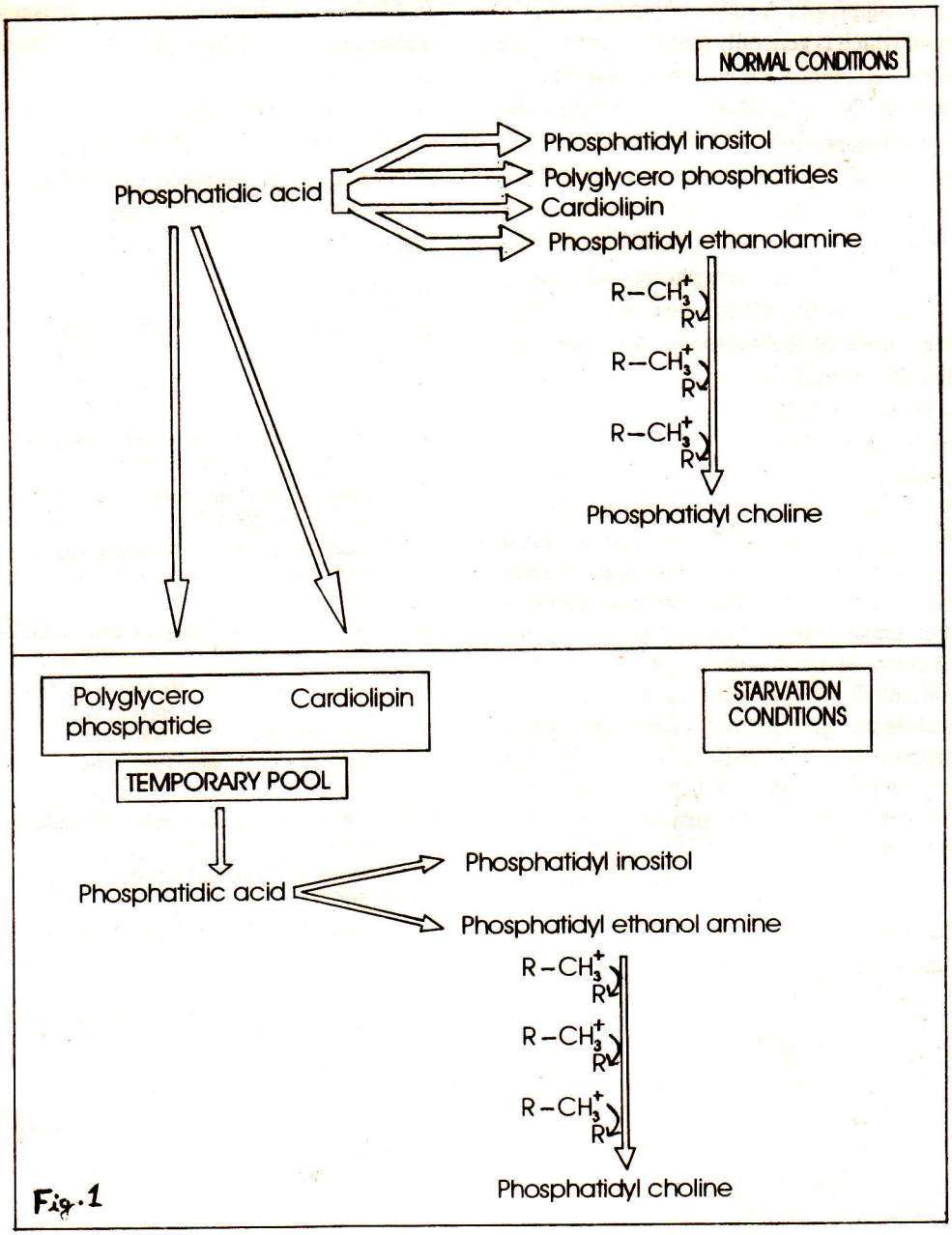

Fig.1 Proposed pathway for the biosynthesis of different phosphatides under normal and starvation conditions.

required for the synthesis of other more indispensable classes required for cell viability, i.e., PC and PE. When the normal growth conditions again prevail, PA synthesis is re-established by the normal process in the cell, then PC and PE are synthesized by the

fresh supply of PA and not from PGP and CL pool which is generally much lower than that observed under the adverse conditions of growth. On replenishing the magnesium and carbon supply to the media, PC again increased while PGP, CL and PA declined (Table 3 and 4) and thus, further supported the above hypothesis. Thus the machinery for the synthesis of the different phosphatides can be switched on and off by altering the cultural conditions of the organism. The increased levels of PGP and CL observed under starvation conditions and their utilization under unfavourable conditions has been explained in Fig.1.

It can be concluded that though the magnesium and carbon starvation inhibited the synthesis of both lipids and proteins significantly, yet such gross variations in the membrane composition did not affect the viability of the membrane, which is being indicated by the great latitude that the membrane showed by a change in relative proportion of its constituents. These studies though preliminary will help to some extent in manipulating the membrane primary components.

Acknowledgements

The authors are highly thankful to United States Department of Agriculture and UNDP-ICAR for providing the financial assistance during the course of this investigation. Thanks are also due to Dr. K L Bajaj, Senior Biochemist and Head, Department of Biochemistry, Punjab Agricultural University, Ludhiana for his valuable suggestions and guidance during the preparation of this manuscript.

References

1. Arneja J S and Sodhi S 1994, *J.Phytol. Res.* **7** 147
2. Chahal D S and Gray W D 1969, *Indian Phytopath.* **22** 79
3. Nomketa C ,Uruburu F and Villanueva L R 1974, *J.Gen. Microbiol.* **81** 247
4. Folch J , Lees M and Sloanestanley G H 1957, *J. Biol. Chem.* **226** 497
5. Stadtman R C 1957, *In: Methods in enzymology*, S. P. Colowik and N. Okaplan, (eds.) Vol.3: Academic Press, New York. p.392.
6. Lowry R R and Tinsley I J 1976, *J.Am. Oil. Chem. Soc.* **53** 470
7. Ames B N 1966, *In: Methods of enzymology*, E.E. Newfed and V. Giinshurg (eds.) Vol. 8, Academic Press, New York. p 115.
8. Arneja J S and Sidhu P 1993, *J. Plant Sci. Res.* **9** 25
9. Gibson K D, Wilson J D and Udenfriend S 1961, *J. Biol. Chem.* **236** 673
10. Bhatia I S and Arneja J S 1978, *J. Sci. Ed. Agric.* **29** 619
11. Arneja J S and Ahuja, J 1992, *J Plant Sci. Res.* **8** 37