A comparison of the fatty acid composition of lecithin and two of its phospholipid precursors from a mutant strain of *Neurospora crassa*

Previous communications from this laboratory[1,2] described the methods for the isolation and identification of phosphatidyl monomethylethanolamine, phosphatidyl dimethylethanolamine and lecithin from a mutant strain of *Neurospora crassa* (strain 47 904). In these studies the above phospholipids were isolated by chromatography on silicic acid columns with mixtures of chloroform and methanol as the eluting mobile phases. The purification of phosphatidyl monomethylethanolamine by rechromatography on a silicic acid column was described in the earlier work[2]. In the present communication phosphatidyl dimethylethanolamine and lecithin were obtained in a sufficiently pure state to make feasible a comparison of the fatty acid moieties contained in the three phospholipids from strain 47 904. Earlier samples of phosphatidyl dimethylethanolamine contained cephalin and trace amounts of phosphatidyl serine as contaminants. Rechromatography of phosphatidyl dimethylethanolamine on a silicic acid column described previously[2] gave the elution pattern shown in Fig. 1. In the present development of this column a large volume of 10% methanol in chloroform (v/v) was employed as the initial phase. The dimethylethanolamine to phosphorus ratios[2] of the column fractions obtained with the subsequent eluant, 15% methanol in chloroform, show that the contaminating phospholipids precede the phosphatidyl dimethylethanolamine and over-lap the first half of this substance coming off the column. The analyses of the fractions eluted after the solvent change to 20% methanol in chloroform have dimethylethanolamine to phosphorus ratios

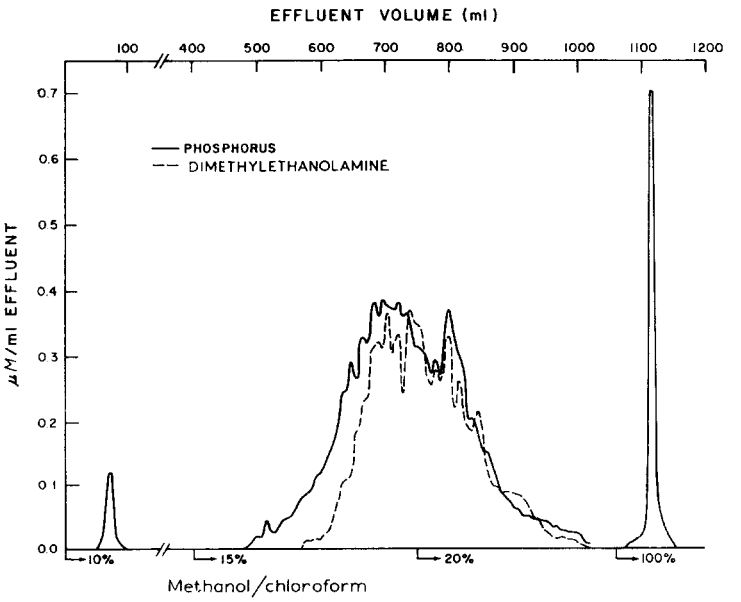

Fig. 1. Rechromatography of phosphatidyl dimethylethanolamine on silicic acid. The column was charged with 88 mg of phospholipid. The curves give values for phosphorus and dimethylethanolamine analyses made on eluate fractions. The horizontal axis indicates solvent changes which are expressed as % of methanol in chloroform (v/v).

which approach the expected values. These fractions were combined and used for the fatty acid analyses (Table I). Two unknown phosphorus containing contaminants were also apparent in the eluates obtained with 10% methanol in chloroform and with 100% chloroform.

TABLE I

FATTY ACID ANALYSES OF PHOSPHOLIPIDS FROM STRAIN 47904*

Length of carbon chain and degree of unsaturation	*Weight % of the total fatty acids released by hydrolysis*		
	Phosphatidyl monomethyl-ethanolamine	*Phosphatidyl dimethyl-ethanolamine*	*Lecithin*
C_{16}	19	18	17
C_{16}–2H**	9	12	7
C_{18}	2	3	5
C_{18}–2H	16	14	15
C_{18}–4H	52	52	54
C_{18}–6H	1	1	1

* These values are for lipids from a 10-day old culture.

** The –2H designation indicates an unsaturated double bond without commitment as to position in chain.

The lecithin in Neurospora strain 47904 represents less than 1% of the total phospholipid. Early attempts in this laboratory to study lecithin samples pooled from several cultures were not successful since it was subsequently observed that the fatty acid composition of phospholipids from different cultures varies even when these are grown under similar conditions. The factors responsible for this variability are not understood. Hence, the present comparison of fatty acids was done on phospholipids obtained from a single culture. Since the choline analyses on hydrolysates of lecithin from strain 47 904 were of necessity on quantities which were at the lower limit of the accuracy of the method[2,3], a direct comparison was made between the unknown sample, taken from the most concentrated fraction of the chromatographic column eluate[2], and an equivalent amount of authentic dipalmitoylphosphatidylcholine*. The molar ratios of recovered choline to phosphorus that were obtained for the unknown and the authentic samples were 0.73 and 0.79, respectively. The relative choline recoveries indicate that the unknown lecithin is better than 90% pure, and on this basis the fatty acid composition of this material is also included in Table I for purposes of comparison.

The fatty acids, released from the phospholipids by mild alkaline hydrolysis[4], were converted to the methyl esters and identified by gas–liquid chromatography. These analyses were done using a Wheelco gas- chromatographic instrument, with a 50-in column of ethylene glycol–succinate polyester on acid-washed chromosorb. The operating temperature was 170°. The results in Table I show the similarity between the fatty acids in the three phospholipids.

It has been proposed that the three substances investigated here represent successive steps in the enzymic methylation of phosphatidyl monomethylethanolamine[2,5–8]. This proposed precursor–product relationship among these phospholipids

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implies that phosphatidyl monomethylethanolamine, phosphatidyl dimethylethanolamine and lecithin can retain the same fatty acid composition during this conversion by methylation if there is no disproportionation of the fatty acid esters in these compounds by competing esterases. The analyses in Table I are in keeping with this conjecture and represent additional evidence for the common metabolic origin of the phosphatidyl moiety in these three phospholipids.

An evaluation of the present literature indicates an alternate pathway for the synthesis of lecithin whereby free choline is formed and subsequently incorporated into lecithin according to the scheme published by KENNEDY[9]. This pathway for the synthesis of lecithin would involve substrates with very different structures from those concerned with the methylation of phosphatidyl monomethylethanolamine. One would not expect the same methylating enzymes to be involved in the two alternate routes concerned with the synthesis of a choline moiety which is common to both paths. On this basis, a mutation at a single locus involving one pathway would not be expected to affect the normal expression of the other metabolic sequence. The Neurospora strain 47 904 lost almost all of its capacity to make lecithin as the result of a single mutation involving the enzyme system which methylates phosphatidyl monomethylethanolamine. A second non-allelic mutant, strain 34486[3,10,11], is known which also cannot make a choline moiety because of a genetic aberration involving one locus. In each of these organisms a single mutation, presumably involving an enzyme common to only one metabolic pathway of choline synthesis, has prevented the formation of most of the lecithin occurring in normal strains. If two or more independent routes of lecithin synthesis are operative in Neurospora, the loss of one metabolic path by mutation would not result in the deficiencies observed in strains 47 904 and 34486. A consideration of these facts, in the light of current concepts of gene–enzyme relationships, indicates that in Neurospora one major pathway exists for lecithin synthesis.

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