Lecithins and Lysolecithins of Wheat Flour[1]

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Abstract

Lecithin and lysolecithin from the bound lipid of Thatcher wheat endosperm were separated and purified by column and thin-layer silicic acid chromatography. Lecithin was hydrolyzed with phospholipase *A (Crotalus adamanteus)* and the products isolated and purified by silicic acid chromatography. The fatty acid composition of the original lecithin and lysolecithin and of the hydrolysis products was determined by gas-liquid chromatography.

The fatty acids in the beta position of the lecithin were found to be almost entirely unsaturated, whereas those in the alpha position were saturated and unsaturated in nearly equal amounts. The differences between the fatty acid composition of the original lysolecithin and that of the lysolecithin obtained by hydrolysis of lecithin with phospholipase *A* suggested the presence of both alpha and beta acyl species in the naturally occurring lysolecithin.

Introduction

THE STRUCTURE OF LECITHINS and their fatty acid distribution have been studied by hydrolysis with phospholipase *A* (1). Earlier work suggested that the ester bond with unsaturated fatty acid was hydrolyzed more readily (2,3). More recent work (4–8) has shown that phospholipase *A* specifically hydrolyzes the ester bond at the beta position. The large proportion of unsaturated fatty acids found in the beta position had given rise to the earlier conclusion that unsaturated acids were preferentially liberated. Definite positional specificity has been established by de Haas and van Deenen (4) who found that only stearic acid was released when synthetic α oleoyl β stearoyl lecithin was hydrolyzed by phospholipase *A* from snake venom *(Crotalus adamanteus)*. Thus phospholipase *A* hydrolysis can be used to determine the positional distribution of fatty acids in lecithin.

The lipids of wheat endosperm have been studied in this laboratory. The free and bound lipids from three different types of wheat were separated into their component classes and the differences within and between classes studied (9). The components of the phospholipids and glycolipids were also separated and compared (10). A study of the fatty acid distribution in lecithin from Thatcher wheat will be reported in this paper.

Experimental and Results

Extraction

Flour was milled (9) from Thatcher wheat. The extraction was carried out in two stages. The free lipids were extracted by shaking 20 g flour with 180 ml cold (1C) deoxygenated hexane and centrifuging at 5 to 15C. The bound lipids were released by shaking the hexane-extracted residue with 180 ml water saturated butanol for 1 hr at 1C in stoppered centrifuge bottles. The suspension was centrifuged for 1 hr at below 15C and the solvent removed from the extract in a rotary evaporator at below 35C. The lipid was kept under nitrogen atmosphere at all times.

Purification and Separation of Lipid Components

The lipid thus extracted was purified by passage through a column of Sephadex (11) to remove nonlipid contaminants. The purified lipid was fractionated by means of silicic acid column chromatography as described by McKillican and Sims (9). The lecithin- and lysolecithin-rich fractions were further purified by TLC using chloroform-methanol-water (65:25:2) as developing solvent. The lipid was then eluted from the silica gel with chloroform-methanol (1:1).

Phospholipase A Hydrolysis

Crotalus adamanteus venom (Ross Allen Reptiles) provided the phospholipase *A* used in this study. Hydrolysis of the lecithin was carried out essentially according to Long and Penny (12). The venom was dissolved in 0.005 M aqueous calcium chloride in the proportion of 1 mg/ml. Fifty microliters of this solution was added to 6 to 8 mg of lecithin in 5 ml of diethyl ether. The hydrolysis was carried out with continuous shaking under nitrogen atmosphere, and was essentially complete in 21 hr. The time necessary for completion of the reaction was determined by monitoring with thin-layer chromatography (TLC). The aliquots of the hydrolysis mixture taken for TLC were developed with chloroform-methanol-water (65:25:2) and the spots revealed by iodine vapor (13) or Dragendorff reagent (14). The reaction was considered complete when no lecithin was detected. When the hydrolysis was complete the mixture was evaporated to dryness under nitrogen and the residue dissolved in anhydrous deoxygenated chloroform. The reaction products were separated on a 1.0 cm I.D. column containing 2 g of 325 mesh silicic acid (Bio-Rad Laboratories). Fractionation was accomplished by solvents of increasing polarity from pure chloroform to pure methanol (15 ml of 100:0, 30 ml of 75:25, 30 ml of 50:50, 15 ml of 25:75, 45 ml of 0:100). The free fatty acids from the column were methylated (15) and the methyl esters and lysolecithin were purified by means of TLC and weighed. The recovery of methyl esters of fatty acids was 3.9 mg per 10 mg lecithin (theoretical 3.7 mg) and of lysolecithin 6.6 mg per 10 mg lecithin (theoretical 6.7 mg).

Fatty Acid Composition

The original lecithin and lysolecithin as well as hydrolysis products were transesterified with boron trifluoride in methanol as described by Morrison (15). The methyl esters were purified by developing with hexane-diethyl ether-acetic acid (90:10:1) on thin-layer plates. The purified methyl esters were eluted from the silica gel with chloroform-methanol and analyzed by gas-liquid chromatography (GLC). A column 6 ft long containing 5% DEGS on Gas Chrom Q was used at 178C in a Research Specialties series 600 gas chromatograph operating with a flame ionization detector. Percentages were calcu-

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lated from calibration curves obtained by the use of an internal standard. As shown in Table I, the principal fatty acids were palmitic, oleic and linoleic acids. The fatty acid composition of the beta-position in lecithin was determined by the fatty acids obtained by hydrolysis. The unsaturated fatty acids amounted to 96.9% and linoleic alone contributed more than 75%. The fatty acid composition of the alpha position was determined in two ways: (a) by difference between the beta position and the total composition of the original lecithin; and (b) by the analysis of the hydrolyzed lysolecithin. As determined by both methods, saturated and unsaturated acids were present in approximately equal amounts. Palmitic acid was the largest single component.

Discussion

The beta position of the lecithin was occupied primarily by linoleic acid. In the alpha position there was slightly more palmitic than linoleic.

The total saturated fatty acid in the beta position was 3.1%, thus the maximum beta-saturated species, i.e. SS + US, in lecithin was 3.1% (US = α unsaturated, β saturated). Similar fatty acid distribution has been found in other plant lecithins. Privett (16) found no SS in soybean lecithin and only a trace in safflower lecithin. In the alpha position the total saturated fatty acid was 59.2% thus the SU species was between 56.1 and 59.2% (SU = β unsaturated, α saturated). Similarly the UU species amounted to between 37.7 and 40.8% of which up to 36.5% could be dilinoleoyl lecithin. A relatively high proportion of UU species is characteristic of plant lecithins; animal lecithins, by contrast, have much lower UU, 2 to 10%.

TABLE I

Fatty Acid Composition
Mole %

Fatty acid	Original		Hydrolyzed lecithin	
	Lecithin	Lysolecithin	Alpha position[a]	Beta position[b]
16:0	25.7	43.7	55.0	3.1
18:0	0.7	0.8	4.2	0
18:1	11.1	5.4	4.3	15.4
18:2	60.5	48.1	36.5	77.4
18:3	2.0	2.0	0	4.1

[a] Lysolecithin obtained by hydrolysis.
[b] Free fatty acids obtained by hydrolysis.

Lysolecithin was shown by TLC to be present in the original wheat endosperm extract before column separations were carried out, and thus was primarily naturally occurring and not a product a transmethylation by methanol on the silicic acid column. The native lysolecithin contained more linoleic and less palmitic than the alpha position of lecithin. Moreover, the native lysolecithin contained 2% linolenic acid whereas none was found in the alpha position of lecithin. Assuming that the fatty acids occur in the same position in native lysolecithin as in lecithin, these differences suggest that the native lysolecithin is a mixture of alpha and beta acyl species. Both species were found by Tattrie (8) in animal lysolecithin. Van den Bosch (17) studied the fatty acid distribution of rat liver lecithin, naturally occurring lysolecithin and lysolecithin produced by phospholipase *A* hydrolysis. He found differences indicating that the beta species amounted to 35% of the naturally occurring lysolecithin.

The lysolecithin in the endosperm lipids of Thatcher wheat thus appears to be a naturally occurring constituent, made up of both alpha and beta species.

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