The Polymorphism of 2-PGMS. The stable form of 2-PGMS at lower temps, called Form I and of DCL appears to be continuous with Form I of 1-PGMS and, hence, with the normal form of a wide range of mixed compositions. The somewhat branched molecular configuration of 2-PGMS apparently favors the tilted structure of Form I. Actually, the diffraction pattern of 2-PGMS from solvent remains that of Form I up to 38C and presumably to the melting point at 40.6C. However, Form I of a melted, chilled sample gives way reversibly to α by 38C. By differential thermal analysis (DTA) the behavior is confirmed, Form I from solvent giving a single sharp peak at the melting level, Form I from melt showing a lower peak for transformation to α in addition to the (α) melting peak. The difference in Form I from solvent and melt is presumed to be a matter of crystal size or degree of perfection.

Just under the melting point, the stable form appears to be α of SCL structure contrasting with DCL for Form I and DCL for α of 1-PGMS, but in this SCL feature, at least, like the SCL structure of Form II of 1-PGMS. The α form of 2-PGMS differs further from the α form of 1-PGMS in lacking the weak, minor short-spacing lines in the diffraction pattern of the latter compound.

The transformation Form I (DCL) $\rightleftharpoons \alpha$(SCL) suggests a major shifting of long chains from a head-to-head DCL structure to some form of SCL structure, either head-to-tail or perhaps with pairing of reversed chains. No macroscopic effects correlated with the change have been observed.

The Binary System 2-PGMS—1-PGMS. It became evident early that much of the complexity in phase behavior of propylene glycol monostearate was due to the content of 2-PGMS in the predominant 1-PGMS isomer. A detailed study of the 2-PGMS—1-PGMS system was strongly indicated. The results of such a study are shown in Table III and Figure 2.

At high 2-PGMS levels, α-1 (α of SCL structure) occurs just below the melting point (after chilling the melt); α-2 (α of DCL structure) occurs similarly on the other side of the diagram. The α melting point curve is continuous, with a sharp break near 50%, the existence of a break corresponding to the occurrence of α-1 for 2-PGMS and α-2 for 1-PGMS. The weakness and diffuseness of the long-spacings in the intermediate region do not permit determination by diffraction technique of the range of existence of the two α phases.

TABLE IV

Comparison of Nomenclatures

M & L[a]	K, B & B[b]
I }	β'
III }	
II	β
α	α

[a] Martin and Lutton.
[b] Kuhrt, Broxholm and Blum.

The stable 1-PGMS phase, Form II, shows a sharp drop in mp on 2-PGMS addition; the phase is difficult to obtain even from solvent with a substantial percentage of 2-PGMS.

The room-temp phase (from melt) is Form I up to 80% or 90% 1-PGMS; above 90% Form III appears (although Form I is still obtainable from solvent) and will transform to Form II, but slowly.

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Composition and Structure of Phospholipids in Chicken Muscle Tissues[1, 2]

C. Y. PENG and L. R. DUGAN, Jr., Department of Food Science, Michigan State University, East Lansing, Michigan

Abstract

Lipids extracted from breast muscle and thigh muscle of one-year old chickens on a standard MSU-Z-4 diet have been fractionated by silicic acid column chromatography into nonphospholipids, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline (lecithin), and sphingomyelins. Phospholipid fractions were identified by thin-layer chromatography and the quantity of each determined by gravimetric analysis, analysis of the phosphorus content, and infrared spectra.

The phospholipid content of thigh muscle (dark meat) lipids was higher than that in the breast muscle (white meat). Phosphatidyl choline and phosphatidyl ethanolamine were found in relatively greater amts than phosphatidyl serine and sphingomyelins. Enzymatic hydrolysis followed by gas-liquid chromatographic analysis of the fatty acids liberated and those in the lysocompounds was used to establish the positional specificity of the fatty acids in the phosphoglycerides. The polyunsaturated fatty acids are located primarily at the β-position and the saturated fatty acids at the α'-position. The qualitative and quantitative determination of the plasmalogens was also accomplished.

[1] Michigan Agriculture Experiment Station Journal Article No. 3527.
[2] Presented at the AOCS Meeting in Chicago, October, 1964.

Introduction

THE PHOSPHOLIPIDS of skeletal muscle have not been studied to the same extent as the phospholipids of organ and neural tissue and those of bacteria. Recent studies on the composition of the phospholipids of avian skeletal muscle have been reported by Davenport (3), and Gray and MacFarlane (5) on pigeon, and by Marion and Woodroof (11) on the broiler. The objective of this study was to investigate the composition and structure of the phospholipids of both dark (thigh) and white (breast) meat in chicken.

TABLE I

Lipid Class Isolated from 100 g Chicken Muscle Tissues

Class	Dark meat		White meat	
	g/100g Sample	%	g/100g Sample	%
Nonphospholipids	1.0917	57.56	0.4413	48.82
Acetone elutable lipids	0.0639	3.37	0.0984	10.89
Phospholipids	0.7409	39.07	0.3642	40.29
Total	1.8965	100.00	0.9039	100.00

Experimental Procedures

Materials. Samples of muscle tissues were taken from two freshly killed one-year-old Cobb strain chickens on a standard MSU-Z-4 diet.

Silicic acid, cp, precipitated, from Fisher Scientific Co., Fair Lawn, N.J., was washed with methanol and dried 24 hr at 120C.

Chromatographic standards for gas-liquid chromatography (GLC) were obtained from California Corporation for Biochemical Research, Los Angeles, Calif.

Crotalus adamanteus venom, obtained from Ross Allen's Reptile Institute, Silver Springs, Fla., was used as a source of phospholipase A.

Silica Gel G, used for thin-layer chromatography, was purchased from Brinkmann Instruments, Inc., Chicago, Ill.

Chromosorb W, acid washed, mesh size 80/100, was obtained from Applied Science Laboratories, Inc., State College, Pa.

All chemicals were analytical reagent grades, and all solvents were freshly redistilled and made suitable for spectrophotometric use.

Lipid Extraction. The procedure for extraction of lipid from muscle tissues was essentially based on the method of Bligh and Dyer (2), modified by adopting the washing system from Folch et al. (4). Nitrogen was used to replace air throughout in extraction and separation while carbon dioxide was used in storage. The final proportion of chloroform, methanol and water was 8:4:3 by volume in the extract. Total lipids were determined by evaporating solvent in vacuo, drying overnight in a vacuum desiccator over calcium chloride, and weighing.

Column Chromatography. A column of activated silicic acid was prepared by pouring 40 g of silicic acid, slurried in an excess of chloroform, into a column (2.2 cm I.D.). The silicic acid was washed with acetone, methanol, and chloroform, respectively, to check whether undesirable channeling existed in the column and to remove colored materials. A 1 cm layer of powdered anhydrous sodium sulfate was then added. Total lipids, redissolved in a small portion of chloroform, were transferred onto the column in a ratio of 0.02 g per 1 g of silicic acid. Elution was accomplished by various solvent systems and successive 35 ml fractions were collected. The flow rate was adjusted to approx 3 ml/min by applying pressure with nitrogen. Nonphospholipids or neutral lipids were eluted by chloroform and monitored by the Salkowski test (9) until negative. Acetone, acting as a scavenger for oxidized materials as suggested by Nelson and Freeman (13), was used to remove pigmented materials. Cephalins were eluted by 15% methanol in chloroform (v/v) and detected in each fraction by ninhydrin. Phosphatidyl ethanolamine was found to come down the column faster than phosphatidyl serine as indicated by a decreasing color intensity of the ninhydrin test. Phosphatidyl choline was eluted by 35%methanol in chloroform (v/v) and its presence confirmed by negative ninhydrin and positive molybdate tests, while sphingomyelins were eluted by 100% methanol and checked by ninhydrin.

Phosphorus Determination. Phosphorus content of each fraction collected was determined by the method of Beveridge and Johnson (1).

Thin-layer Chromatography. Thin-layer absorption chromatography on Silica Gel G was used to check the identities of phospholipids (9). All components were applied in a solution of chloroform or chloroform-methanol. The developing solvent used for separation was chloroform: methanol:water (65:25:4) by volume (20). Lipid component spots were detected with ninhydrin solution for amino phosphatides, molybdic acid for phosphatides, and Dragendorf reagent for choline (17). The relative R_f values were 0.79 for phosphatidyl ethanolamine, 0.54 for phosphatidyl serine, 0.64 for phosphatidyl choline, and 0.48 for sphingomyelins.

Infrared Spectra Analysis. All infrared (IR) spectra determinations have been made with a Beckman IR-5 double beam recording Spectrophotometer equipped with a sodium chloride prism, and an absorption cell with an optical path length of 0.0992 mm. All readings were taken from strong absorption bands; therefore, phosphatidyl ethanolamine and phosphatidyl serine were at 5.8 μ because they have been shown to exhibit similar (19) or identical (18) IR spectra, phosphatidyl choline at 10.3 μ and sphingomyelins were at 6.1 μ.

Hydrolysis. Phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl choline were subjected to enzymatic hydrolysis in an aqueous medium at 37C, pH 7.3, with glycylglycine buffer and sodium deoxycholate (10) with phospholipase A in snake venom from *Crotalus adamanteus* (6). Sphingomyelins were hydrolyzed with sulfuric acid (12). The liberated free fatty acids, the unhydrolyzed phospholipides, and the lysocompounds were separated on a silicic acid column by chloroform, 25% methanol in chloroform (v/v), and 100% methanol, respectively. The unhydrolyzed phospholipids and the lysocompounds were subjected to basic hydrolysis with 0.5 M methanolic KOH (8).

Gas-Liquid Chromatography. The fatty acid composition of all phospholipid components was determined quantitatively by GLC of the methyl esters

TABLE II

Phospholipid Components Isolated from 100 g Chicken Muscle Tissues

Component	Dark meat				White meat					
	Phosphorus content		Gravimetry		Phosphorus content		Gravimetry		IR spectra	
	g/100g	%	g/100g	%	g/100g	%	g/100g	%	g/100g	%
PE[a]	0.2303	30.02	0.1833	24.74	0.0652	15.40	0.0563	15.46	0.0573	12.16
PS[b]	0.0681	8.88	0.0519	7.00	0.0394	9.30	0.0330	9.06	0.0279	5.92
PE and PS[e]	0.0429	5.59	0.0467	6.30	–	–	–	–	–	–
PC[c]	0.3992	52.04	0.4305	58.11	0.2457	58.01	0.2243	61.59	0.3100	65.80
PC and SP[e]	–	–	–	–	0.0594	14.03	0.0403	11.06	0.0706	14.99
SP[d]	0.0266	3.47	0.0285	3.85	0.0138	3.26	0.0103	2.83	0.0053[f]	1.13
Total	0.7671	100.00	0.7409	100.00	0.4235	100.00	0.3642	100.00	0.4711	100.00

[a] Phosphatidyl ethanolamine.
[b] Phosphatidyl serine.
[c] Phosphatidyl choline.
[d] Sphingomyelin.
[e] Fraction in which both components were present as determined by TLC.
[f] Determined on sample from which phosphoglycerides had been removed by mild basic hydrolysis.

in an F and M Model 500 temperature programmed gas chromatograph (F and M Scientific Corporation, Avondale, Pa.) equipped with a thermal conductivity detector, Honeywell Electronik recorder, and the Series 200 DISC integrator. A coiled copper column (7.5 ft by ¼ in. O.D.) was used for methyl ester separation. It was packed with 20% by weight DEGS (Lac-728, F and M Scientific Corporation, Avondale, Pa.) and 1% by weight phosphoric acid on acid-washed chromosorb W, 80/100 mesh, as a support phase. The operating conditions used in this study were: Column temp, 210C; injection port temp, 265C; detector block temp, 250C; carrier gas, helium; carrier gas flow rate, 80 ml/min; reference gas flow rate, 120 ml/min; attenuator setting, 2; bridge current, 150 ma; chart speed, 80 sec/in. Identifications of the fatty acids on the chromatogram were made by comparing the semilog plots of retention times vs. carbon numbers of the chicken lipid methyl esters with those of known mixtures of methyl esters run on the same column under the same conditions. The fatty acid compositions were expressed as area percentage of the total area from all methyl esters.

Results and Discussion

The presence of phosphatidyl ethanolamine, phosphatidyl serine, and sphingomyelins were readily determined by the ninhydrin test. Phosphatidyl choline detection gave some trouble but a satisfactory method resulted from a combination of Beveridge's method for phosphorus (1) and Skidmore's determination of phosphatides (17). This involved adding 0.05 ml of concd sulfuric acid to 0.1 ml of eluate and heating two min at 130–140C in a sand bath, after which 0.05 ml of molybdic acid was immediately added. A blue color developed if phosphatides were present. The intensity of the blue color was a function of the concn of phosphatidyl choline in the sample. Therefore, the phosphatidyl choline fraction was identified with a negative ninhydrin test and a positive molybdate test.

Table I shows the lipid classes isolated from 100 g chicken muscle tissues by column chromatography. The total lipids in dark meat are twice those in the white meat; however, the percentage distribution of phospholipids in both is about the same at 40%. Table II compares three methods, phosphorus content determination, gravimetric analysis, and IR spectra analysis, which have been used to determine each component contained in white meat, and two methods, phosphorus content determination and gravimetric analysis, to determine each component in dark meat phospholipids. There is more of each phospholipid component on a weight basis in the dark meat than that of its counterpart in the white meat. This is probably due to more exercise by thigh than breast muscle. Phosphatidyl choline and phosphatidyl ethanolamine are present in relatively greater amts than the phosphatidyl serine and sphingomyelins; however, there are some discrepancies. The main difference is in the amt of sphingomyelins determined by IR spectra analysis when compared to the amt of sphingomyelin determined by the other methods. This is similar to the observations made in a study of pork muscle phospholipids by Kuchmak and Dugan (9). Since sphingomyelin is resistant to basic hydrolysis due to the amide linkage (15), one sample, containing phosphoglycerides and sphingomyelin from white meat, was purified by subjecting it to basic hydrolysis. The resulting products were separated on a silicic acid column and checked for purity of sphingomyelin by TLC. The sphingomyelins thus purified were determined by IR spectra and gave a value of 0.0053 g/100 g. This value was probably more precisely representative than those obtained for sphingomyelin by other analyses.

TABLE III
Absorption Coefficients

Component	Solvent	Wavelength (μ)	Absorbance per mg per ml (a)	Molar[a] absorptivity (ϵ)
PE	CS_2	5.8	0.908	67656
PS	$CHCl_3$	5.8	0.671	53175
PC	$CHCl_3$	10.3	0.449	36008
PC	$CHCl_3$	5.8	0.773	61666
SP	$CHCl_3$	6.1	0.596	44019

[a] Assuming following relative mol wts: phosphatidyl ethanolamine, 743; phosphatidyl serine, 787; phosphatidyl choline, 786; and sphingomyelins, 728.

Table III lists a set of absorption coefficients of the measured phospholipid standards at their principal absorption band. The absorption coefficients, designated a, absorbance per mg per ml, and ϵ, molar absorptivity, were calculated by Lambert-Beer's Law. Since different materials have definitely different absorption coefficients due to different fatty acid composition and location (16), it was deemed preferable to prepare our own standards from chicken muscle tissues by chromatography. Solvents selected for this study were based principally on the solubility of the various lipid classes in them. Hence, phosphatidyl ethanolamine was measured in carbon disulfide, while phosphatidyl serine, phosphatidyl choline and sphingomyelins were measured in chloroform. It should be emphasized that these values are specific only for the material, cells, and instruments used in this study.

The plasmalogens were first detected qualitatively by the reaction of mercuric chloride with an α,β-unsaturated ether and the color developed with diphenyl carbohydrazide (14). Phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl choline gave positive tests for the presence of plasmalogens while sphingomyelins were negative. Plasmalogen content shown in Table IV was determined quantitatively by the uptake of iodine because of the sensitivity of the method and the linear relationship between iodine uptake and sample size (21). The relative mol wt of plasmalogen was calculated on the basis of linoleic acid and stearyl aldehyde attached to the β- and α'-positions, respectively. Thus, the mol wt of plasmalogen in the phosphatidyl ethanolamine form is 727, it is 771 in the phosphatidyl serine form and 770 in the phosphatidyl choline form. Plasmalogens in chicken muscle tissues are most prevalent in the phosphatidyl choline form and least prevalent in the phosphatidyl serine form.

After enzymatic or basic or acid hydrolysis of phospholipid components, all liberated free fatty acids were converted to methyl esters by the method of Hornstein et al. (7). GLC analysis of the methyl esters derived from dark meat phospholipids is shown in Table V and that from white meat phopholipids in Table VI. In dark meat the predominant fatty acid in phosphatidyl ethanolamine is stearic acid. At the α'-position the chief component, stearic acid, is accompanied by lesser amts of linoleic, oleic and

TABLE IV
Percentage of Plasmalogens in Each Component

Component	Dark meat	White meat
Phosphatidal ethanolamine	3.51	5.19
Phosphatidal serine	1.00	2.50
Phosphatidal choline	13.19	9.97

TABLE V

Fatty Acid Composition of Phosphatidyl Ethanolamine, Phosphatidyl Serine, Phosphatidyl Choline, and Sphingomyelins in Dark Meat (Area %)

FA [a]	PE			PS			PC			SP
	α'-FA	β-FA	Unhydrolyzed [b]	α'-FA	β-FA	Unhydrolyzed [b]	α'-FA	β-FA	Unhydrolyzed [b]	
6:0	–	–	–	–	–	–	–	–	–	Trace
8:0	–	–	2.09	–	–	–	–	–	–	Trace
9:0	–	–	–	–	–	–	6.69	1.91	1.51	–
10:0	–	–	–	–	–	–	5.18	2.19	7.52	1.61
11:0	–	–	–	–	–	–	4.11	1.06	–	2.29
12:0	3.78	2.37	1.91	1.54	4.89	3.25	3.16	2.95	4.09	2.91
?	–	0.51	–	–	–	–	–	–	–	–
13:0	1.05	–	0.97	1.86	4.00	6.66	–	1.20	2.00	2.67
?	1.08	–	–	–	–	–	–	–	–	–
14:0	2.24	1.25	2.16	1.12	4.44	2.03	–	1.16	1.56	9.73
14:1	–	–	–	–	–	–	–	–	1.27	–
?	1.75	0.77	1.32	–	–	–	–	–	–	–
15:0	3.00	0.80	2.36	1.08	3.77	3.82	–	1.74	1.01	2.60
16:0	6.84	5.16	6.61	3.08	9.99	4.71	19.50	9.23	21.18	9.73
16:1	2.62	1.68	2.78	–	–	–	–	–	–	1.67
17:0	2.12	0.79	1.63	2.00	3.89	3.41	–	–	–	–
17:1	–	–	–	–	–	–	–	0.62	1.89	–
?	1.95	–	–	–	–	–	–	–	–	–
18:0	35.79	17.22	58.61	59.88	18.32	9.67	21.07	4.21	18.67	11.78
18:1	10.68	13.51	10.08	12.95	18.82	19.17	7.77	29.60	14.36	16.12
18:2	12.54	22.25	9.48	14.18	16.10	16.90	3.85	35.60	14.25	5.92
18:3	0.90	2.06	–	1.39	2.22	1.18	–	–	–	–
20:0	–	–	–	0.92	–	–	–	–	–	–
20:1	–	–	–	–	–	–	0.40	0.37	2.69	–
21:0	3.20	–	–	–	–	–	–	–	1.36	0.62
20:4	10.47	31.63	–	–	2.89	1.22	–	8.17	3.46	8.49
22:1	–	–	–	–	5.55	19.85	–	–	1.12	2.51
23:0	–	–	–	–	3.89	–	14.12	–	1.05	7.44
24:0	–	–	–	–	–	–	14.17	–	1.03	–
24:1	–	–	–	–	1.22	8.12	–	–	–	4.12
25:1	–	–	–	–	–	–	–	–	–	9.67

[a] Fatty acids. [b] Unhydrolyzed refers to unchanged portion of sample separated after enzymatic hydrolysis.

arachidonic acids; while arachidonic acid is the main component at the β-position, with linoleic, stearic and oleic acids in decreasing order. The question marks indicate components for which no standard was available to identify them on the chromatogram. The main fatty acids contained in phosphatidyl serine are stearic, oleic, linoleic, docosaenoic and tetracosaenoic acids. At the α'-position, the chief acids are stearic, oleic and linoleic acids while oleic, stearic, linoleic and palmitic are the main fatty acids at the β-position. Palmitic acid is predominant in phosphatidyl choline, followed by stearic, oleic and linoleic acids. Saturated fatty acids, stearic, palmitic, tricosanoic and lignoceric, are found mainly at the α'-position while the unsaturated linoleic, oleic and arachidonic acids predominate at the β-position. Sphingomyelins contain chiefly oleic, and stearic acids, followed by lesser amts of myristic, palmitic, arachidonic, pentacosaenoic and tricosanoic acids.

Since the structure pattern was determined in dark meat, only basic hydrolysis was carried out on the white meat components. It is probable that the positional distribution of the fatty acids is the same in phosphatides from either source. The major fatty acid of phosphatidyl ethanolamine is stearic acid, which was also the major fatty acid in phosphatidyl ethanolamine in the dark meat, while arachidonic acid is the next. This differs from the observations made on dark meat phosphatidyl ethanolamine. Phosphatidyl serine contains mainly oleic acid which agrees with its composition in dark meat, then cerotic, arachidonic and stearic are the next most commonly found fatty acids. Palmitic acid is the predominant fatty acid in phosphatidyl choline in white meat as well as in dark meat; oleic, linoleic, stearic and arachidonic acids follow. In the sphingomyelin molecule oleic acid is present in the greatest amt, the same as in dark meat; next are undecanoic, linoleic and myristic acids.

The predominant fatty acid contained in phosphatidyl ethanolamine is stearic acid. Oleic acid is the main acid in both phosphatidyl serine and sphingomyelins, while palmitic acid is dominant in phosphatidyl choline. These observations hold both in dark and white muscle tissues. Some variations of amts of other fatty acids do exist. The polyunsaturated fatty acids are located primarily at the β-position and the saturated fatty acids at the α'-position as has been observed in phopholipids from other sources.

TABLE VI

Fatty Acid Composition of Phosphatidyl Ethanolamine, Phosphatidyl Serine, Phosphatidyl Choline, and Sphingomyelins in White Meat (Area %)

FA	PE	PS	PC	SP
10:0	1.30	3.62	2.88	9.35
10:1	–	2.57	2.15	–
11:0	–	–	–	10.33
11:1	0.70	2.51	2.10	–
12:0	1.16	–	–	6.79
13:0	4.29	1.46	8.75	6.57
14:0	–	1.52	–	9.76
15:1	2.60	0.64	1.73	5.19
16:0	7.51	1.69	30.20	7.78
16:1	–	–	1.63	6.67
17:0	–	0.70	–	–
17:1	–	1.34	–	3.68
18:0	58.10	10.21	9.65	6.36
18:1	7.72	42.54	18.24	13.15
18:2	4.31	1.52	12.21	10.14
18:3	0.15	–	–	–
20:4	12.15	10.91	9.38	4.23
24:1	–	1.28	1.07	–
25:0	–	0.64	–	–
25:1	–	5.52	–	–
26:0	–	11.32	–	–

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