Distribution of Membrane Phospholipids in the Crystalline Lens

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Purpose. To determine the phospholipid content of specific anatomic regions within the crystalline lens.

Methods. Phospholipid extracts of tissues dissected from 5 sets of 10 rabbit lenses were analyzed by ^{31}P nuclear magnetic resonance spectroscopy. Twenty-nine pathway-specific metabolic indexes were calculated from groups of phospholipids and ratios of phospholipids.

Results. Phospholipid levels (mole percent) were determined from the capsule with attached epithelium, the cortex, and the nucleus. Eleven phospholipids were detected with significant regional differences in the lens phospholipid profiles. The levels of phosphatidylcholine (PC), PC plasmalogen–alkylacyl PC, phosphatidylinositol (PI), phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG), and of the lyso derivatives (lyso PC and lyso PE) were greater in the capsule plus epithelium than in the cortex or the nucleus. Levels of sphingomyelin, phosphatidylserine, and PE plasmalogen (EPLAS) were less in the capsule plus epithelium than in the cortex or the nucleus. PC, PC plasmalogen–alkylacyl PC, EPLAS, and lyso PE had nearly equal amounts in the cortex and the nucleus. PI, lyso PC, and DPG could not be detected in the nucleus. DPG was only detected in the capsule plus epithelium. An unidentified phospholipid at 0.13 ppm was approximately equal in the cortex and the nucleus, but it could not be detected in the capsule plus epithelium.

Conclusions. These differences demonstrate a significant heterogeneity among these anatomic regions of the lens, and differences in the nucleus relative to other regions studied are consistent with those in membranes that less readily undergo transitions from the relatively impermeable lamellar phase to the more permeable hexagonal H_{II} phase. Invest Ophthalmol Vis Sci. 1994;35:3739–3746.

Quantitative analysis of phospholipids within the crystalline lens can be conducted using ^{31}P nuclear magnetic resonance (NMR) spectroscopy.[1,2] This technique has limitations in that the analyses to date have been conducted without knowledge of the contribution of the various regions of the lens, the capsule plus epithelium, the cortex, and the nucleus, to the whole-lens spectrum. Knowledge of the regional distribution of specific phospholipids would provide improved interpretation based on anatomic regions of the lens affected by cataractogenesis. The present study uses lenses dissected into three anatomically distinct regions. The phospholipids of each of these anatomic zones were quantitated and compared to each other and to the whole-lens phospholipid profile.

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MATERIALS AND METHODS

Surgical

Eyes were harvested from rabbits (each weighing 4 to 5 kg) immediately after administration of a lethal dose of ketamine. Sagittal and horizontal incisions were made at the posterior pole. Curved blunt scissors were used to extend these incisions to the ora serrata. The vitreous humor was gently separated, and the lens was exposed. Zonules were cut with curved scissors. Lenses were dissected according to methods previously de-

scribed.[3,4] Briefly, dissection was conducted with the use of a Zeiss (Oberkochen, Germany) Operating Microscope and the lens capsule plus epithelium was peeled from the cortex and immediately immersed in liquid nitrogen. The lens cortex was separated from the lens nucleus, and both tissues were immersed immediately in liquid nitrogen. Whole lenses were not dissected. Five lenses were used for each of five separate experiments. The investigations using animals conform to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Extraction Procedure and Sample Preparation

A simple modified Folch[5] extraction of the lens phospholipids was performed in which the backwashing step uses potassium (ethylenedinitrilo)-tetraacetic acid (EDTA), 0.2 M in EDTA, pH 6.0.[6] Lens samples frozen in liquid nitrogen were pulverized to a fine powder with a stainless steel mortar and pestle chilled with liquid nitrogen. The homogeneous tissue powder (average, 1 g per lens sample) was then added to 20 ml of chloroform–methanol (2:1, vol/vol). The homogenate, exhibiting only one liquid phase, was filtered into a separatory funnel. The extract was washed with a 4-ml volume of 0.2 M KEDTA and allowed to separate thoroughly for 24 hours in a separatory funnel. The chloroform phase was recovered and evaporated using a rotary evaporator at 37°C. The analytical medium for ^{31}P NMR phospholipid analysis was a hydrated chloroform–methanol NMR reagent[7,8] specifically designed for the quantitative[6–9] determination of phospholipids by ^{31}P NMR.

^{31}P Nuclear Magnetic Resonance Spectroscopy

A multinuclear GE 500NB spectrometer system (Fremont, CA) operating at 202.4 MHz for ^{31}P was employed. Tissue extract samples were analyzed in standard 10-mm NMR sample tubes during 21-hour periods. Analysis was performed using proton broad-band decoupling to eliminate ^{1}H-^{31}P NMR multiplets.

Chemical-shift data in units of ppm are reported relative to the usual standard of 85% inorganic orthophosphoric acid.[10–12] The primary internal standard was the naturally occurring phosphatidylcholine (PC) resonance at −0.84 ppm.[7] Identification of resonance signals,[3,7,13,14] quantification of those signals,[3,6–9] and spectral curve resolution[2,15] were performed according to methods previously described.

Spectrometer analytical scan parameters were as follows: pulse sequence, one pulse; pulse width, 18 μsec (45° spin–flip angle); acquisition delay, 500 μsec; interpulse delay, 500 msec; number of acquisitions, 50,000; free-induction decay size, 2,000 channels; acquisition time, 1.02 sec; sweep width, 1000 Hz. An exponential multiplication time-constant introducing 0.6-Hz computed line broadening was applied to enhance spectral signal-to-noise ratios.

Phospholipids Determined and Metabolic Indexes Calculated

The following phospholipids were detected: lysophosphatidylethanolamine (LPE), diphosphatidylglycerol (cardiolipin) (DPG), an uncharacterized phospholipid (U), ethanolamine plasmalogen (EPLAS), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM), lysophosphatidylcholine (LPC), phosphatidylinositol (PI), choline plasmalogen or alkylacylphosphatidylcholine (CPLIP), and PC.

In addition to measuring differences in tissue phospholipid composition, the analyses include metabolic indexes computed from groups of phospholipids, ratios of phospholipids and ratios of groups of phospholipids. These indexes compare and contrast phospholipids and provide more pathway-specific metabolic interrelations. The indexes are mathematical devices useful for interpreting metabolic profile data. They are not an absolute measure, and, further, they may not be supported by known metabolic processes. For example, the OUTSIDE/INSIDE ratios were inferred from erythrocytes and viruses. These have not been measured directly in ocular tissues, and it is known from other tissues that some fraction of PC and SM may exist on both sides of plasma membranes. The use of these indexes does not imply that all PC, for example, resides in the outer membrane leaflet.

The computed metabolic indexes for the phospholipids are defined as follows: OUTSIDE, PC+SM; INSIDE, PE+PS; LEAFLET, OUTSIDE/INSIDE[16]; SM/PC; SM/PE; SM/EPLAS; SM/PS; PC/PE; PC/EPLAS; PC/PS[17]; PE/PS; EPLAS/PS; PLASA, CPLIP+EPLAS; PLASB, PC+PE; UNSAT, PLASA/PLASB; UNSATC, CPLIP/PC; UNSATE, EPLAS/PE; LECITHIN, PC+CPLIP; CEPHALIN, PE+EPLAS; LECITHIN/CEPHALIN; CHOLA (all choline-containing phospholipids except the lyso derivatives), PC+CPLIP+SM; CHOLB (all other phospholipids except the lyso derivatives), PI+PS+PE+ EPLAS+DPG; CHOLINE, CHOLA/CHOLB; ANIONIC/ NEUTRAL (ratio of anionic phospholipids to neutral–ionic phospholipids), (DPG+PS+PI)/(EPLAS+PE+SM+CPLIP+PC); LYSO, LPE+LPC; NLYSO, DPG+EPLAS+PE+PS+SM+PI+PC; LYSO/NLYSO; LPC/PC; LPE/PE. Rationales for the use of these indexes have been discussed.[1,15,18–22]

Data Reduction and Statistical Analysis

Phospholipid concentrations were determined through integration of the phospholipid resonance signals detected from each of the tissue specimen extracts. The relative mole fraction of each signal con-

tributing to a given spectral profile was then calculated as a percentage of the total spectral integral. Absolute quantitation of phospholipids was precluded by the variability inherent in the analytical methods employed for such an extraction. Subsequent to the calculation of the relative phospholipid concentration values, the phospholipid metabolic indexes were calculated for each tissue specimen. From these computed relative phospholipid concentrations, mean relative mole percentages were calculated[23] for all resonances that were detected in all specimens of each lens tissue fraction: capsule plus epithelium, cortex, and nucleus. Similarly, tissue means were computed for all metabolic indexes constructed from the phospholipid relative mole percentages.

Initially, the three tissue groups were compared at the level of the individual phospholipid or index values by an analysis of variance. For those mean phospholipid or index values where significance was determined to exist (F probability, < 0.05), post hoc simple contrasts were applied. Simple contrasts employed the Scheffé comparison procedure, with $P < 0.05$ accepted as significant. Under most conditions, analysis of variance requires the assumption that the underlying variances between tested means are equal. For those resonances where significance was found to exist, homogeneity of variance was confirmed using Cochran's C and the Bartlett–Box F tests.[23]

RESULTS

^{31}P NMR spectral profiles of the separate lens regions studied (capsule plus epithelium, cortex, and nucleus) are presented, along with values obtained from undissected (whole) lens tissue, in Table 1. The NMR spectra (Fig. 1) illustrate the similarities and differences among the 11 phospholipids detected. Minor resonances detected in this study are LPE, DPG or cardiolipin, and LPC. Two uncharacterized phospholipids (U, CPLIP) also were detected.

Examination of the phospholipid NMR spectral profiles (Fig. 1) reveals that the most notable differences among the profiles are quantitative: The capsule plus epithelium does not contain detectable quantities of the uncharacterized phospholipid at 0.13 ppm, whereas the cortex and the nucleus do not contain detectable quantities of DPG. Further, LPC and PI could not be detected in the nucleus. Other notable findings include the following: A high level of LPE in the capsule plus epithelium, which is approximately three times greater than those values in either cortex or nucleus. A slightly higher level of the uncharacterized phospholipid at 0.13 ppm in the cortex relative to the nucleus. EPLAS values in the cortex and nucleus that are approximately twice that in the capsule plus epithelium. EPLAS also is slightly greater in the nucleus relative to the cortex. PE is nearly three times as concentrated in the capsule plus epithelium and twice as concentrated in the cortex relative to the nucleus. PS is reduced in the capsule plus epithelium relative to the cortex and nucleus, which exhibit the same concentration. The concentration of SM is least in the capsule plus epithelium, greater in the cortex and approximately twice as concentrated in the nucleus. Both LPC and PI are more than three times as concentrated in the capsule plus epithelium than in the cortex but are not detectable in the nucleus, as mentioned previously. CPLIP decreases slightly in the following order: capsule plus epithelium, cortex, nucleus. PC is greatest in the capsule plus epithelium relative to both the cortex and the nucleus, where the concentrations are equal.

The data of Table 1 are presented as the mole fraction in each of the phospholipid extracts, respectively. The contribution of each of the regions to the whole-lens phospholipid spectrum is modified by the fact that the total tissue mass in each of the three regions, as well as the phosphatide concentration within each region, is not identical. Whole (intact) lens data also are presented in Table 1 for comparison. The whole-lens data reflect the bulk lens tissues, which are the cortex and the nucleus.

DISCUSSION

The present study introduces evidence consistent with the hypothesis that the phospholipids from each lens region contribute to the total lens phosphorus spectrum proportionately to the amounts of each phospholipid in each tissue fraction. Thus, the whole-lens spectral profile represents the weighted arithmetic sum of the three contributing tissue profiles. For example, consider the five phospholipids characteristic of most mammalian tissues: EPLAS, PE, PS, SM, and PC.[2,15,19–22,24–28] These phospholipids also account for the largest proportion of the total lens phospholipid profile. As in most eye tissues, PC is the most concentrated phospholipid, although there are substantial concentrations of EPLAS, PE, PS, and SM.[2,8,15,18,24,25] The capsule plus epithelium exhibits a phospholipid profile distinctly different from that of the cortex or the nucleus. Because the capsule plus epithelium contributes a small weight-fraction to the total lens mass, however, the whole (intact) lens profile reflects the weight-fractions of the cortex and the nucleus, which account for the bulk of the lens tissue. The whole-lens profile, however, exhibits small quantities of DPG, a phospholipid unique to the capsule plus epithelium, and the amount is in the range that might be anticipated from the weight-fraction contribution of capsule plus epithelium to the whole lens. These data compare favorably with date of an earlier report on lens phos-

TABLE 1. ^{31}P Nuclear Magnetic Resonance Phospholipid Profiles of Rabbit Lens Capsule + Epithelium, Cortex, Nucleus, and Whole Lens

		Mole % Detected Phospholipid (Means ± SD)			
Phospholipid	*Chemical Shift (ppm)*	*Capsule + Epithelium*	*Cortex*	*Nucleus*	*Whole Lens*
LPE	0.43	1.58 ± 0.16	0.40 ± 0.10*	0.56 ± 0.13†‡	0.61 ± 0.28
DPG	0.18	4.51 ± 0.26	···*	···†	0.31 ± 0.29
U	0.13	···	6.86 ± 0.28*	5.78 ± 0.20†‡	4.99 ± 0.35
EPLAS	0.11	11.22 ± 0.46	20.10 ± 0.28*	24.28 ± 0.58†‡	21.29 ± 0.84
PE	0.06	16.31 ± 0.16	12.16 ± 0.44*	5.77 ± 0.55†‡	12.22 ± 0.31
PS	−0.05	8.95 ± 0.27	13.52 ± 0.36*	13.09 ± 0.12†‡	11.54 ± 0.57
SM	−0.09	8.86 ± 0.31	12.24 ± 0.50*	18.55 ± 0.16†‡	13.58 ± 0.55
LPC	−0.27	1.54 ± 0.16	0.56 ± 0.25*	···†‡	0.46 ± 0.51
PI	−0.33	3.93 ± 0.24	1.30 ± 0.17*	···†‡	0.50 ± 0.37
CPLIP	−0.78	2.88 ± 0.16	2.22 ± 0.25*	1.86 ± 0.21†‡	2.62 ± 0.15
PC	−0.84	40.22 ± 0.86	30.64 ± 0.46*	30.09 ± 0.61†	31.88 ± 0.56

LPE = Lysophosphatidylethanolamine; DPG = diphosphatidylglycerol (cardiolipin); U = uncharacterized phospholipid; EPLAS = ethanolamine plasmalogen; PE = phosphatidylethanolamine; PS = phosphatidylserine; SM = sphingomyelin; LPC = lysophosphatidylcholine; PI = phosphatidylinositol; CPLIP = choline plasmalogen or alkylacylphosphatidylcholine; PC = phosphatidylcholine.

* Lens cortex compared to lens capsule + epithelium ($P < 0.05$).

† Lens nucleus compared to lens capsule + epithelium ($P < 0.05$).

‡ Lens nucleus compared to lens cortex ($P < 0.05$).

pholipids[2] in which comparisons between the ^{31}P NMR method of phospholipid analysis and other traditional methods were discussed.[1,2]

Table 1 reports concentrations of phospholipids in units of mole percent, which is a relative concentration measurement. Attempts also were made to quantitate the absolute contribution of each dissected lens tissue's phospholipid complement, so that a calculation could be performed documenting the proportionality of each tissue's contribution to the total lens phospholipid profile from phospholipid moles-per-unit-weight data. Attempts at absolute quantitation failed because of factors introducing variability in the analytical methods that must be employed for absolute quantitation. For example, an important factor is the requirement that an exhaustive phospholipid tissue extraction be performed rather than simply a representative extraction. Exhaustive extractions of organic materials using organic solvents cannot be performed with the requisite precision. There are two additional sources of error. One is biologic variability.[6,8,9] The other is that extracted phospholipids are not chemically pure substances; rather they are of generic biologic composition and represent families of closely related molecules. (This chemical reality requires that quantitation be based on the phosphorus heteroatom, the only chemical factor that is unique to all of the phospholipids regardless of the variation in their fatty-acid composition.) These error sources render absolute phospholipid quantitation problematic regarding tissues. The semiquantitative profiles obtained through the use of ^{31}P NMR on simple Folch[5] extracts have proven reliable because such determinations demand only that a representative sample be obtained. Such representative extracts can be prepared using a minimum of chemical procedures, and they yield relative concentration values of high precision. The entire issue of quantitation in ^{31}P NMR and the use of mole percent in presenting quantitative data has been analyzed in detail by Klunk et al.[29]

Table 1 lists the concentrations of the constituent lens phospholipids, whereas Table 2 presents metabolic indexes that are combinations of the phospholipid values presented in Table 1. These indexes define metabolic systems and provide more refined pathways for discussion. Particularly noteworthy are the ratios of the inner membrane leaflet[16] phospholipids (PS, PE, EPLAS, and, perhaps, phospholipid U[26], if the initial interpretation of this unknown proves correct) to the outer leaflet membrane phospholipids (SM, PC, and, perhaps, CPLIP). The computed metabolic indexes relevant to this feature are: OUTSIDE, INSIDE, LEAFLET, SM/PC, SM/PE, SM/EPLAS, SM/PS, PC/PE, PC/EPLAS, PC/PS, PE/PS, EPLAS/PS. The capsule plus epithelium is enriched in the outer leaflet components, whereas the cortex is enriched in the inner leaflet components. The differences are consistent with those in capsule membranes less permeable to the translocation of ions, solutes, and proteins relative to that of the cortex. This interpretation is consistent with the known resistance of the lens capsule to the translocation of solutes.

Phosphatidylethanolamine in consort with its plasmalogen (and, perhaps, phospholipid U[26]) promotes

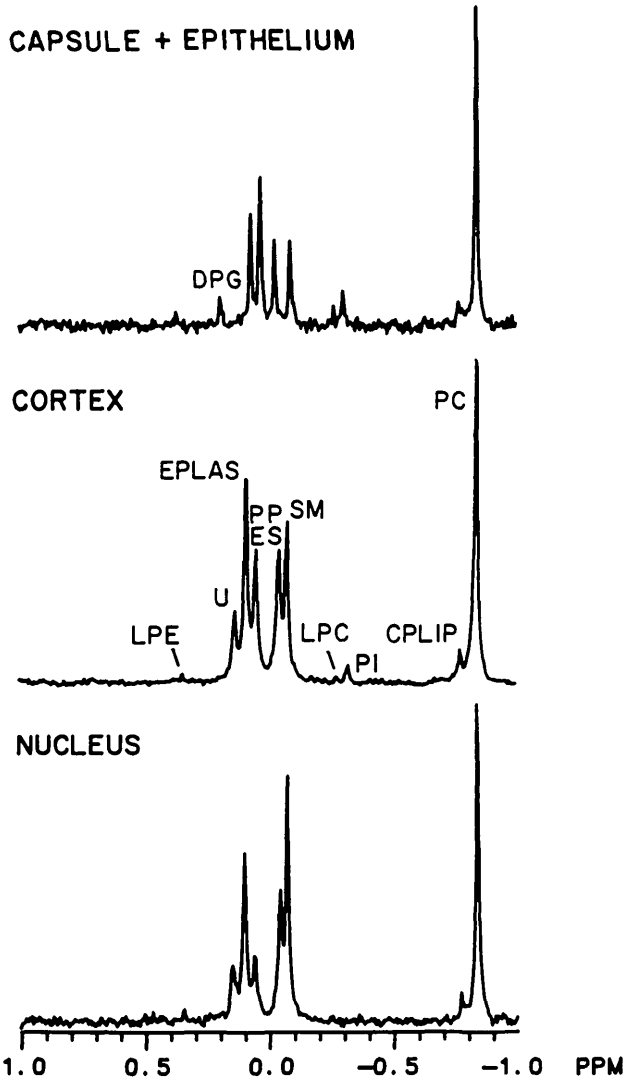

FIGURE 1. ^{31}P NMR membrane phospholipid profiles of the rabbit lens capsule plus epithelium, cortex, and nucleus: diphosphatidylglycerol (cardiolipin) (DPG); lysophosphatidylethanolamine (LPE); uncharacterized phospholipid (U); ethanolamine plasmalogen (EPLAS); phosphatidylethanolamine (PE); phosphatidylserine (PS); sphingomyelin (SM); lysophosphatidylcholine (LPC); phosphatidylinositol (PI); choline plasmalogen (or alkylacylphosphatidylcholine) (CPLIP); phosphatidylcholine (PC). The PC resonance is off-scale so that the more minor resonances may be observed.

membrane transition from the lamellar form to the hexagonal H_{II} form or the intermediate lipidic particle form.[30,31] In these forms—the transitions to which are often promoted by certain peptides[32] and by the signal peptide sequences of certain proteins[33]—and in contrast to the lamellar form, the membrane becomes permeable to the translocation of ions, organic molecules, and even proteins.[33]

The notable features of the nucleus phospholipid profile compared to the cortex phospholipid profile are that the nucleus profile is reduced in PE, there is a compensatory enhancement in EPLAS, and SM is enhanced. This is reflected by indexes PLASA and PLASB, the ratio indexes UNSAT, UNSATC, and UNSATE, and the values of the choline–ethanolamine indexes LECITHIN, CEPHALIN, LECITHIN/CEPHALIN, CHOLA, CHOLB, and CHOLINE. The enhancement of the plasmalogen over its diacyl analogue may reflect a more reducing environment in the nucleus relative to that of the cortex in which formation of the more chemically reduced plasmalogen enol-ether is favored. The enhancement of SM by 6%, with no compensatory change in PC phospholipids, suggests that nuclear membranes may be less permeable to ion translocation by a modest degree than are cortex membranes (indexes SM/PC, SM/PE, SM/EPLAS, SM/PS). This change is consistent with the known elevated biochemical activity of the cortex relative to those of both the capsule and the nucleus.

The PI concentration is relatively low for mammalian tissue,[7,19–22,26–28] even for the tissues of the ocular tunica fibrosa,[15,18] but it is consistent with low PI levels detected in lenses of other classes.[1,2,8,24,25,34] This results in a relatively low index value for ANIONIC/NEUTRAL in all three tissue segments; however, this index, which is a measure of membrane net negative charge, is lowest in the nucleus and highest in the capsule.

Lysophospholipids are detrimental to the viability of cellular membranes, and it is possible that the values obtained for LPC and LPE in these experiments may result from breakdown during the lens lipid preparation procedures. Such degradation to the lyso compounds has not, however, been observed using synthetic phospholipid preparations, standardized preparations from a variety of natural sources,[19–22,26–28] or tissue preparations to which synthetic phospholipids have been added.[7,14,26] The lysophospholipid compounds, which are always observed at very low levels, appear to be genuine natural constituents of many biologic sources. LPC is particularly ubiquitous.[1,2,6–8,15,18–22,26–28,34] For example, LPC is a well-documented constituent of all circulating lipoproteins.[35–37]

The lysophospholipid metabolic indexes are LYSO, NLYSO, the ratio index LYSO/NLYSO, which proportions the net lyso compliment to that of the net parent phospholipid, and the specific ratio indexes LPC/PC and LPE/PE. The cortex and nucleus have very low LYSO/NLYSO ratios. The cortex value is half that of the capsule plus epithelium, and the nucleus value is only one fifth that of the capsule plus epithelium. The low value for this ratio in the nucleus reflects a stable phospholipid composition with little turnover that proceeds through the lysophospholipid derivative.

A feature of the ^{31}P NMR method is its ability to detect previously undetected and unknown phospho-

TABLE 2. ^{31}P Nuclear Magnetic Resonance Phospholipid Indexes of Rabbit Lens Capsule + Epithelium, Cortex, and Nucleus

	Index Value (Means ± SD)		
Index	*Capsule + Epithelium*	*Cortex*	*Nucleus*
OUTSIDE	49.07 ± 0.63	42.88 ± 0.13*	48.63 ± 0.53‡
INSIDE	25.25 ± 0.12	25.68 ± 0.22	18.85 ± 0.54†‡
LEAFLET	1.94 ± 0.033	1.67 ± 0.014*	2.58 ± 0.955†‡
SM/PC	0.221 ± 0.012	0.400 ± 0.022*	0.616 ± 0.016†‡
SM/PE	0.543 ± 0.020	1.007 ± 0.033*	3.240 ± 0.327†‡
SM/EPLAS	0.791 ± 0.052	0.609 ± 0.024*	0.764 ± 0.017‡
SM/PS	0.992 ± 0.042	0.906 ± 0.051*	1.417 ± 0.001†‡
PC/PE	2.47 ± 0.042	2.52 ± 0.109	5.26 ± 0.568†‡
PC/EPLAS	3.59 ± 0.166	1.52 ± 0.039*	1.24 ± 0.046†‡
PC/PS	4.50 ± 0.212	2.27 ± 0.068*	2.30 ± 0.062†
PE/PS	1.826 ± 0.073	0.901 ± 0.055*	0.441 ± 0.043†‡
EPLAS/PS	1.25 ± 0.039	1.49 ± 0.026*	1.86 ± 0.039†‡
PLASA	14.10 ± 0.37	22.32 ± 0.51*	26.13 ± 0.41†‡
PLASB	56.53 ± 0.97	42.80 ± 0.46*	35.86 ± 0.60†‡
UNSAT	0.250 ± 0.009	0.522 ± 0.018*	0.729 ± 0.022†‡
UNSATC	0.072 ± 0.005	0.073 ± 0.009	0.062 ± 0.006
UNSATE	0.688 ± 0.032	1.65 ± 0.072*	4.26 ± 0.473†‡
LECITHIN	43.10 ± 0.77	32.86 ± 0.36*	31.95 ± 0.75†
CEPHALIN	27.53 ± 0.40	32.26 ± 0.40*	30.04 ± 0.54†‡
LECITHIN/CEPHALIN	1.56 ± 0.036	1.02 ± 0.024*	1.06 ± 0.043†
CHOLA	51.96 ± 0.58	45.10 ± 0.23*	50.49 ± 0.65†‡
CHOLB	44.91 ± 0.61	47.08 ± 0.26*	43.12 ± 0.59†‡
CHOLINE	1.157 ± 0.027	0.958 ± 0.005*	1.171 ± 0.031‡
ANIONIC/NEUTRAL	0.219 ± 0.007	0.192 ± 0.005*	0.163 ± 0.002†‡
LYSO	3.127 ± 0.324	0.957 ± 0.282*	0.608 ± 0.128†
NLYSO	93.99 ± 0.48	89.96 ± 0.25*	91.75 ± 0.23†‡
LYSO/NLYSO	0.033 ± 0.004	0.011 ± 0.003*	0.007 ± 0.001†
LPC/PC	0.038 ± 0.005	0.018 ± 0.008*	0.000 ± 0.000†‡
LPE/PE	0.097 ± 0.010	0.033 ± 0.009*	0.106 ± 0.020‡

Index definitions: OUTSIDE (PC + SM); INSIDE (PE + PS); LEAFLET (OUTSIDE/INSIDE); PLASA (CPLIP + EPLAS); PLASB (PC + PE); UNSAT (PLASA/PLASB); UNSATC (CPLIP/PC); UNSATE (EPLAS/PE); LECITHIN (PC + CPLIP); CEPHALIN (PE + EPLAS); CHOLA (all choline-containing phospholipids except the lyso derivatives) (PC + CPLIP + SM); CHOLB (all other phospholipids except the lyso derivatives) (PI + PS + PE + EPLAS + DPG); CHOLINE (CHOLA/CHOLB); ANIONIC/NEUTRAL [(DPG + PS + PI)/(EPLAS + PE + SM + CPLIP + PC)]; LYSO (LPE + LPC); NLYSO (DPG + EPLAS + PE + PS + SM + PI + PC).
PC = Phosphatidylcholine; SM = sphingomyelin; PE = phosphatidylethanolamine; PS = phosphatidylserine; CPLIP = choline plasmalogen or alkylacylphosphatidylcholine; EPLAS = ethanolamine plasmalogen; PI = phosphatidylinositol; DPG = diphosphatidylglycerol; LPE = lysophosphatidylethanolamine; LPC = lysophosphatidylcholine.
* Lens cortex compared to lens capsule + epithelium ($P < 0.05$).
† Lens nucleus compared to lens capsule + epithelium ($P < 0.05$).
‡ Lens nucleus compared to lens cortex ($P < 0.05$).

lipids, such as phospholipids CPLIP and U (Table 1). The specific chemical identities of CPLIP and U are not known. CPLIP has been identified both as the choline plasmalogen,[7] which contains the enol-ether functional group at the glycerol 1-carbon position, and as the corresponding alkylacyl–phosphatidylcholine,[26] which can be thought of as a derivative of the choline plasmalogen that has been further reduced to an alkyl function at the glycerol 1-carbon position. A corresponding analogue of the platelet activating factor (alkenylacetylPC), the alkylacetylPC, is known to be an active biochemical[38]; however, neither compound is a proven constituent of lens tissue.

It is suspected[26] that the phospholipid labeled U is the corresponding alkylacylphosphatidylethanolamine, but this assignment is even less certain than those for the corresponding choline phospholipids. It should be noted, however, that the assignment of resonance U to the alkylacylphosphatidylethanolamine is consistent with data reported from this laboratory regarding the chemical and spectroscopic properties of an unknown phospholipid at 0.13 ppm in eye tissues,[1,2,15,18,34] as well as in other human tissues.[19,20,21,28] In the human lens, phospholipid U has been detected at very elevated concentrations.[1,34] In earlier work involving the human lens,[24,25] U had been incorrectly assigned as SM, which is quite low in concentration in the human lens.[1,34]

The CPLIP and U ^{31}P NMR spectral assignments are not certain because of the inability, thus far, of producing chemically pure grade samples of both compounds that are not cross-contaminated. The newly recognized phospholipids, however, have characteristic and unique chemical shifts in the ^{31}P NMR spectrum that permit their secure spectral recognition and precise quantitation in lens tissue spectroscopic phospholipid profiles.

The uncharacterized phospholipid U is noticeably absent in the capsule, whereas DPG is absent in the cortex and the nucleus but is present in the capsule plus epithelium. The relative concentrations of these two phospholipids in their respective profiles are approximately the same. Perhaps DPG may play the role in the capsule plus epithelium attributable to U in the cortex and the nucleus.

Key Words

crystalline lens, phospholipids, plasmalogens, capsule, epithelium, cortex, nucleus, ^{31}P, nuclear magnetic resonance (NMR)

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