Sphingomyelin Inhibits the Lecithin-Cholesterol Acyltransferase Reaction with Reconstituted High Density Lipoproteins by Decreasing Enzyme Binding*  

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Lecithin-cholesterol acyltransferase (LCAT) catalyzes the formation of cholesterol esters on high density lipoproteins (HDL) and plays a critical role in reverse cholesterol transport. Sphingomyelin, an important constituent of HDL, may regulate the activity of LCAT at any of the key steps of the enzymatic reaction: binding of LCAT to the interface, activation by apo A-I, or inhibition at the catalytic site. In order to clarify the role of sphingomyelin in the regulation of the LCAT reaction and its effects on the structure of apolipoprotein A-I, we prepared reconstituted HDL (rHDL) containing egg phosphatidylcholine, cholesterol, apolipoprotein A-I, and up to 22 mol % sphingomyelin. Because the interfacial properties of substrate particles can dramatically affect LCAT binding and kinetics, we also prepared and analyzed proteoliposome substrates having the same components as the rHDL, except for a 4-fold higher ratio of phospholipid to apolipoprotein A-I. The reaction kinetics of LCAT with the rHDL particles revealed no significant change in the apparent Vmax, but showed a concentration-dependent increase in slope of the reciprocal plots and in the apparent Km values with sphingomyelin content. The dissociation constant (Kd) for LCAT with these particles increased linearly with sphingomyelin content up to 22 mol %, changing in parallel with the apparent Km values. No structural changes of apolipoprotein A-I were detected in the particles with increasing content of sphingomyelin, but fluorescence results with lipophilic probes revealed that significant changes in the acyl chain, backbone, and head group regions of the lipid bilayer of the particles are introduced by the addition of sphingomyelin. On the other hand, the proteoliposome substrates also had increased Kd values for LCAT at high sphingomyelin contents but compared with the rHDL particles had a 6-10-fold lower affinity for LCAT binding and exhibited kinetics consistent with competitive inhibition by sphingomyelin at the active site. These results show conclusively that the dominant mechanism for the inhibition of LCAT activity with rHDL particles by sphingomyelin is the impaired binding of the enzyme to the interface. The results also underscore the significant differences in the enzyme reaction kinetics with different substrate particles.

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The abbreviations used are: LCAT, lecithin-cholesterol acyltransferase; CE, cholesterol ester; PC, phosphatidylcholine; HDL, high density lipoproteins; apo A-I, apolipoprotein A-I; rHDL, reconstituted HDL; SPM, sphingomyelin; DPPC, dipalmitoyl-PC; DMPC, dimyristoyl-PC; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammonium phenyl)-6-phenyl-1,3,5-hexatriene; PRODAN, 6-propanoyl-2-(dimethylaminophenyl)naphthalene.
has an altered epitope expression compared with discoidal rHDL particles, suggesting that the altered lipid composition of lymphatic HDL promotes an apoA-I conformation that may render it incapable of activating LCAT (11). Clearly, structural and compositional differences of lymphatic HDL contribute to their lower reactivity with LCAT. The phospholipid content of HDL could dramatically alter LCAT activity by interacting with and changing the conformation of apoA-I, by changing the nature of the lipid interface, by inhibiting LCAT through competition for substrate binding at the active site, or by a combination of the above. It is possible that the presence of SPM in HDL alters LCAT reactivity by several of these mechanisms.

In addition to its effects in native lipoproteins, SPM has been shown to be a poor matrix for the LCAT reaction with synthetic substrates. In fact, LCAT activity is lower with PC regardless of the acyl chain composition when the PC is presented in an SPM matrix compared with a fluid ether PC matrix (12). Research by Subbaiah and Liu (13) using proteoliposome substrates and native lipoproteins suggests that SPM competes with PC for binding to the active site of LCAT and thus participates in the regulation of the LCAT reaction. Although proteoliposomes are useful as substrates for LCAT in vitro, they lack the defined apoA-I structures characteristic of discoidal reconstituted HDL (rHDL) and have very different structures from native HDL. In order to clarify the role of SPM in the regulation of the LCAT reaction and its effects on the structure of apoA-I, we have prepared discoidal rHDL with egg PC, cholesterol, and apoA-I containing up to 22 mol % SPM. We have characterized these particles in terms of protein structure and properties of the lipid components and the lipid-water interface. We examined the structure of apoA-I and the binding affinity and reaction kinetics of LCAT with these particles. Our results suggest that in discoidal rHDL particles SPM introduces changes in the structure of the lipids, decreases the binding of LCAT to the substrate particles, and thus regulates the LCAT reaction. In contrast, proteoliposome substrates bind more weakly to LCAT and experience the regulatory effect of SPM not only at the binding step but also at the catalytic step.

**EXPERIMENTAL PROCEDURES**

Materials and Preparations—Human LCAT was purified by methods described previously (14, 15). Its average specific activity, using standard rHDL substrates, was around 100 nmol CE/hr/μg LCAT, and it remained fully active over an 8-month period. Human apoA-I was prepared using a modification of the method of Nichols et al. (16). Egg PC, egg SPM, cholesterol, and sodium cholate were obtained from Sigma. Radiolabeled [4-14C]cholesterol and [3H]-labeled [2-palmitoyl-9,10-H]palmitoylphosphatidylcholine (3H-DPPC) were purchased from DuPont NEN.

The rHDL were prepared using the sodium cholate dialysis method (17) in molar ratios of 80:8:1:80, egg PC/cholesterol/apoA-I/cholate, or in ratios ranging from 74:(4):1:80 to 51:(17):8:1:80 when prepared with SPM (given in parenthesis). Proteoliposome preparations contained 312:5:1:936 molar ratios of egg PC/cholesterol/apoA-I/cholate, or ratios of 275:(35):5:1:936 and 234:(78):5:1:936 when containing SPM (in parenthesis). Radiolabeled cholesterol (5,000 cpm/nmol) was incorporated only into the particle preparations that were used for the determination of reaction kinetics with LCAT. Radiolabeled [3H]-DPPC (20,000 cpm/nmol of cholesterol) was included in the preparation of the standard substrate for the activity inhibition measurements (18). Cholate was removed by exhaustive dialysis against 0.1 M Tris-HCl, 0.005% EDTA, 0.15 NaCl, 1 mM NaHCO3, pH 8.0 buffer at 4 °C. Diameters of rHDL were measured by nondenaturing 8–25% polyacrylamide gradient gel electrophoresis (Pharmacia PHAST gradient gel electrophoresis). Phosphatidylcholine was separated from SPM and cholesterol by TLC (AnalaR Analytical) using chloroform/methanol/ammonia, v/v/v and quantitated using the method of Chen et al. (19). Protein content was determined from absorbance at 280 nm using the percentage extinction coefficient for apoA-I, 11.5 × 10^4 g/cm^2 (20) and by the method of Lowry et al. (21).

Egg PC/C or SPM/C vesicles were prepared in ratios of 10:1, phospholipid/cholesterol. Preparations were dried down and dispersed in 10 ml of standard buffer. The samples were sonicated on ice (egg PC) or at 50 °C (SPM) until they cleared, alternating 3 min of sonication with 3-min rest periods. The vesicles were centrifuged at 15 °C for 1 h. The phospholipid content of the supernatant (~5 mg/ml) was assessed by the method of Chen (19) and with a standard phospholipid assay kit (Wako Phospholipids B). The vesicles were used immediately following the preparation.

**Activity Inhibition Assay for Determining the Binding of LCAT—**

**LCAT affinity for the rHDL and proteoliposome particles was assessed by activity inhibition assay previously described (18). Briefly, the reaction mixture included 3H-DPPC and egg rHDL preparations containing labeled apoA-I. Unlabeled apoA-I or apoA-I liposomes were added to the reaction mixture. The reaction was quenched and processed as previously described (18).**

Fluorescence Characterization and Circular Dichroism—**The lipid dynamics and hydration of the rHDL containing SPM were examined using fluorescent probes. The motions and polarity of the environment of the acyl chain, glycerol backbone, and head group regions were assessed using 1,3,5 diphenylhexatriene (DPH), trimethylammonium-DPH (TMA-DPH), and 6-propionyl-2-dimethylaminonaphthalene (PRODAN), respectively. All fluorescent lipophilic probes were obtained from Molecular Probes (Eugene, OR). Fluorescence measurements and analysis of the data were performed as described previously (25). Circular dichroism spectra were measured with a Jasco J-720 spectro-
TABLE I
Characterization of the rHDL particles

| SPM \( \text{mol} / \text{mol} \) | Composition (A-I/C/PC/SPM) \( \text{mol} / \text{mol} / \text{mol} / \text{mol} \) | Diameter \( \AA \) | \( \alpha \)-Helicity \( \% \) |
|---|---|---|---|
| 0 | 1/8/75/0 | 97 | 71 |
| 3.7 | 1/8/74/4 | 97 | 74 |
| 11 | 1/8/69/10 | 97 | 72 |
| 17 | 1/8/60/14 | 97 | 70 |
| 22 | 1/8/51/17 | 97 | 71 |

*PC was separated from SPM by TLC (Analtech Analytical) using chloroform/methanol/ammonia (65:25:4, v/v/v) and quantified using the method of Chen et al. (19). Cholesterol content from the initial phospholipid/cholesterol ratios. Protein content from absorbance at 280 nm and extinction coefficient. The errors of measurement are approximately \( \pm 5 \% \).

RESULTS
ApoA-I has been shown to combine with a variety of phospholipids, including SPM, to form stable discoidal rHDL particles (12, 28–30). Table I summarizes the properties of rHDL particles prepared in this study; their size distribution is shown in Fig. 1. The composition and size of the rHDL particles are consistent with a discoidal morphology (31). The moderate content of egg SPM, with saturated acyl chains (86% palmitoyl) (32), does not appear to alter significantly the structure of apoA-I. Circular dichroism spectra for all of the rHDL in this series were quite similar (data not shown) indicating that the \( \alpha \)-helical content of apoA-I changes very little, as shown in Table I. It is clear that rHDL particles with similar size and total lipid contents can be prepared with apoA-I and mixtures of egg PC and SPM. The proteoliposome preparations had protein and lipid compositions very similar to those of the initial reaction mixtures and migrated on nondenaturing gradient gel electrophoresis as heterogeneous populations of particles most having diameters greater than 180 \( \AA \) (data not shown).

LCAT reactivity with rHDL is highly dependent upon the phospholipid composition of the interface of the substrate particle. SPM provides a poor matrix for the LCAT reaction, as shown in Fig. 1. The proteoliposome substrates had increasing slopes for the highest SPM contents (Fig. 2A) consistent with increasing \( K_d \). However, the app\( V_{\text{max}} \) values decreased with added SPM, suggesting competition of SPM for PC at the active site. These results are in complete agreement with those reported by Subbaiah and Liu (13). The apparent kinetic constants are summarized in Table II. In the absence of the SPM inhibitor, the app\( V_{\text{max}} \) is 37\% higher for an rHDL than for a proteoliposome substrate, and the app\( K_m \) (in terms of PC concentration) is 4.4-fold lower for rHDL, giving an overall 5.2-fold greater catalytic efficiency (app\( V_{\text{max}} / \text{app} K_m \)) for the rHDL substrates. This is the first quantitative comparison of these two widely used synthetic substrates for LCAT.

To confirm that SPM increases the \( K_d \) for the interaction of rHDL and proteoliposomes with LCAT, we measured directly the LCAT binding affinity of a series of rHDL and proteoliposome particles without \( ^{14} \text{C}- \text{cholesterol} \) using the activity inhibition assay previously described (18). We found that the \( K_d \) for rHDL increased about 5-fold in a linear manner with increasing SPM, and the \( K_d \) for proteoliposomes increased 2-fold (see Table III). Clearly, the presence of SPM in the particles decreases LCAT affinity for the phospholipid interface. To determine if the effect of SPM on LCAT binding affinity could be observed independently of apoA-I, we prepared vesicles with cholesterol and either egg PC or SPM in a 1:10 molar ratio of cholesterol to phospholipid. The vesicles were used as test particles in competition with the standard LCAT substrate rHDL under the same conditions as the activity inhibition assay. As the amount of vesicle phospholipid increases, LCAT binds to the vesicle surface, and as a result, net production of \( ^{14} \text{C}- \text{cholesterol ester} \) at the standard substrate surface decreases. Fig. 3 shows that the amount of SPM vesicle phospholipid step are in effect constants, then \( K_d \) is the likely variable that affects the slope of the Lineweaver-Burke plots shown in Fig. 2.
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Fig. 2. Lineweaver-Burke plots of LCAT reaction kinetics with proteoliposomes (A) or rHDL (B) containing SPM. ■, 22 mol % SPM; ○, 17 mol % SPM; ●, 11 mol % SPM; □, 5 mol % SPM; ▲, 0 mol % SPM. Initial reaction velocities (v) were measured at 37 °C using reaction mixtures containing substrate concentrations ranging from 8.0 × 10⁻⁶ M to 5.0 × 10⁻⁴ M PC, 2 mg of defatted bovine serum albumin, 4 mM β-mercaptoethanol, and 30–50 ng of pure LCAT in 10 mM Tris-HCl, 0.15 M NaCl, 0.01% EDTA, 1 mM NaN₃, pH 8.0. Substrate rHDL contained 5,000 cpm [4-¹⁴C]cholesterol/nmol cholesterol. Two separate experiments on two preparations were performed in duplicate; each gave similar results.

Table II

| Substrates          | SPM (mol %) | Kₐ⁻⁻ (M, A-I) | Kₐ⁻⁻ (M, PC) | appVₘₐₓ (nmol CE/h)⁻¹ | appKₘₐₓ (M, PC)⁻¹ | appVₘₐₓ/appKₘₐₓ (nmol CE/h/M⁻¹) |
|---------------------|-------------|---------------|--------------|-----------------------|------------------|---------------------------------|
| rHDL                | 0           | ×10⁻⁸         | ×10⁻⁹        | 15.7                  | 3.2              | 49.0                            |
|                     | 3.7         | 6.0           | 4.4          | 15.0                  | 4.0              | 37.5                            |
|                     | 11          | 8.0           | 5.5          | 15.7                  | 5.0              | 31.4                            |
|                     | 17          | 11.0          | 6.4          | 17.0                  | 6.4              | 26.6                            |
|                     | 22          | 14.0          | 6.9          | 15.7                  | 8.2              | 19.1                            |
| Proteoliposomes     | 0           | 7.9           | 24.0         | 11.5                  | 14.0             | 8.2                             |
|                     | 11          | 14.0          | 34.0         | 6.8                   | 7.6              | 8.9                             |
|                     | 23          | 19.0          | 41.0         | 3.4                   | 5.7              | 6.0                             |

* The dissociation constants were determined by the activity inhibition method as described under "Experimental Procedures" using the molar concentrations of apoa-I (A-I) or PC in the calculations. Average of two experiments. Errors shown on Figure 3.

** The apparent kinetic constants were determined from Lineweaver-Burke analysis of initial velocity versus molar PC concentrations using rHDL particles containing [4-¹⁴C]cholesterol. Initial reaction velocities were measured at 37 °C in 10 mM Tris, 0.15 M NaCl, 0.01% EDTA, 1 mM NaN₃, pH 8.0. The results are expressed in terms of PC concentrations. Two separate experiments were performed in duplicate; each gave similar results.

The phospholipid (0.25 mg/ml) required to inhibit the LCAT reaction by 50% was nearly 5-fold higher than the amount of egg PC vesicle phospholipid (0.048 mg/ml) necessary for a similar inhibition. This result indicates that LCAT binds to the SPM/cholesterol vesicle surface in the absence of apoA-I with less affinity than it does to the egg PC/cholesterol vesicle surface. Because the Kₐ for LCAT binding to the egg PC rHDL particles is 2.3 × 10⁻⁵ M or 0.018 mg/ml (Table II), it follows that the affinity of LCAT for the rHDL is about 3-fold greater than for the egg PC/cholesterol vesicles. Table II also shows that the affinity of LCAT for rHDL in the absence of SPM is 10-fold greater than for comparable proteoliposomes.

To investigate the effects of the addition of SPM on the properties of the rHDL phospholipid phase, we examined the lipid dynamics and hydration of the surface of the rHDL particles using lipophilic fluorescent probes. DPH fluorescence polarization reports on the fluidity of the acyl chain region of the rHDL particles. Fig. 4 shows the temperature dependence of the polarization of DPH for the rHDL containing 22% SPM, 11% SPM, and 0% SPM. DPH polarization increases with increasing SPM content in the rHDL. As shown in Fig. 5, changes in TMA-DPH polarization with temperature indicate similar effects in the phospholipid backbone region, the region between the hydrophobic acyl chains, and the hydrophilic head group region. The higher polarization values observed with both probes indicate that the mobility of the lipids is restricted and order is increased.

The fluorescence of PRODAN was used to probe the polarity of the phospholipid head group region. The fluorescence spectra of PRODAN are quite sensitive to the polarity of the probe environment (33, 34). Fig. 6 shows the fluorescence intensity ratio for PRODAN at 440/490 nm. The probe is in a more polar environment in the egg PC control rHDL compared with the rHDL containing SPM as indicated by the blue-shift that occurs with increased SPM content in the rHDL. This suggests that the presence of SPM shields PRODAN from water molecules in the head group region. These changes are consistent with decreased hydration of egg PC/SMP interfaces as a result of altered phospholipid packing or hydrogen bonding of SPM to cholesterol or to PC, which displaces water (35) and/or allows the probe to penetrate more deeply into the head group region.

DISCUSSION

Much of what is known about the regulation of LCAT activity with lipoprotein substrates has come from kinetic studies of LCAT with substrate analogs, such as proteoliposomes or rHDL. Many factors have been shown to modulate LCAT activity in vitro with these substrates. Among these are substrate size and morphology (disc versus sphere), phospholipid composition (head group, acyl chain, and unsaturation), and apolipoprotein composition and conformation (36). It is clear that these factors are closely interrelated. As a consequence, exam-
ining the effect of one of these parameters on LCAT activity while holding the others constant is a difficult task; however, rHDL prepared by the sodium cholate dialysis method have made possible detailed studies of the LCAT reaction with particulate substrates of defined apolipoprotein and phospholipid compositions and similar morphologies.

The conformation of apoA-I, the principal physiological activator of LCAT, is one of the key determinants of LCAT activity. ApoA-I conformation is related to the size of the particle and the phospholipid composition and content. In terms of the number of \( \alpha \)-helices/molecule, apoA-I may adopt a conformation with six, seven, or eight \( \alpha \)-helices, depending upon the amount of lipid complexed with the protein. But not all confor-

**FIG. 3.** Inhibition of LCAT activity by phospholipid vesicles. Vesicles of egg PC/cholesterol (▲) or SPM/cholesterol (■) were prepared by sonication in ratios of 10:1, phospholipid/cholesterol. Reaction conditions were identical to those of the activity inhibition method. Concentrations of vesicle phospholipid in the assay mixture ranged from \( 1.3 \times 10^{-3} \) to 3.2 mg/ml.

**FIG. 4.** Effects of temperature on DPH fluorescence polarization in rHDL containing SPM. ▲, 22 mol % SPM rHDL; ●, 11 mol % SPM rHDL; ▲, 0 mol % SPM rHDL. DPH was added to rHDL samples (0.1 mg/ml apoA-I) in the ratio 300:1, phospholipid/probe (mol/mol). Polarization values were obtained using a ISS GREG PC photon counter spectrophotometer using the following parameters: excitation wavelength, 366 nm; emission wavelength, 430 nm; slit width, 8 nm. Each point is the average of five measurements. Two separate experiments were performed giving similar results.

**FIG. 5.** Effects of temperature on TMA-DPH fluorescence polarization in rHDL containing SPM. ▲, 22 mol % SPM rHDL; ●, 11 mol % SPM rHDL; ▲, 0 mol % SPM rHDL. TMA-DPH was added to rHDL samples (0.1 mg/ml apoA-I) in the ratio 300:1, phospholipid/probe (mol/mol). Polarization values were obtained as described in the legend to Fig. 4, except that an emission filter at 400 nm (Corning, KV399) was used. Each point is the average of five measurements. Two separate experiments were performed giving similar results.

**FIG. 6.** PRODAN fluorescence intensity ratio at 440/490 nm in SPM containing rHDL as a function of temperature. ▲, 22 mol % SPM rHDL; ●, 11 mol % SPM rHDL; ▲, 0 mol % SPM rHDL. PRODAN was added to rHDL samples (0.1 mg/ml apoA-I) in the ratio 300:1, phospholipid/probe (mol/mol). Experiments were performed as described previously (26). Two separate experiments were performed giving similar results.
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Miyazawa, N., et al.

The authors discuss the role of Sphingomyelin (SPM) in the inhibition of LCAT (Lecithin-Cholesterol Acyltransferase) activity. They explain that the presence of SPM in the proteoliposomes can significantly alter the binding and reactivity of LCAT, as evidenced by changes in the reaction kinetics and the determination of the Michaelis-Menten constants (Km, Vmax) for LCAT. The data suggest that SPM competes with phosphatidylcholine (PC) at the active site of LCAT, leading to a decrease in the reaction rate (Vmax) and an increase in the dissociation constant (Km). These effects are observed across a range of SPM concentrations, indicating a dose-dependent inhibition.

The authors also provide evidence that SPM alters the physical properties of the proteoliposomes, which in turn affect LCAT activity. Specifically, they show that changes in the hydration and packing of the lipid bilayer, induced by SPM, can modify the enzymatic activity of LCAT. This is supported by changes in fluorescence properties of probes such as TMA-DPH and DPH, which are used to monitor the lipid order and organization in the proteoliposomes.

Furthermore, the authors demonstrate that the inhibition of LCAT by SPM is not due to the presence of SPM at the active site, but rather occurs at an earlier step in the reaction sequence, possibly due to changes in the initial binding of LCAT to the proteoliposomes. They conclude that SPM's role in modulating LCAT activity is significant and provides insights into the mechanism of its inhibition, which could have implications for conditions such as hypercholesterolemia and cardiovascular disease.
The effects of SPM on the head group region are also significant. The presence of SPM facilitates close lipid packing and condensed organization of the head group region due to its hydrogen bonding capacity. The results of $^{31}$P NMR in egg PC/SPM vesicles suggest that intramolecular hydrogen bonds of SPM and close lipid packing may partially exclude water molecules from hydrating the phosphate group of PC, resulting in decreased hydration of the head group region (35). This would explain the relative blue shift of PRODAN fluorescence in rHDL with increased SPM content.

During the revision of this paper a study by Rye et al. (45) was published on the effects of SPM on the structure and function of spherical and discoidal rHDLs. Rye and co-workers demonstrated that SPM affects the lipid order and packing in these particles, in close agreement with our observations. They reported that SPM does not influence neutral lipid transfers involving spherical rHDL and cholesterol ester transfer protein; they also showed that SPM inhibits LCAT reaction with rHDL substrates. However, Rye et al. did not address the mechanism of LCAT inhibition by SPM, which is the main topic of this report.

In summary, we report that a SPM content up to 22 mol % does not alter the size of rHDL prepared with bulk egg PC, cholesterol, and apoA-I. LCAT binding affinity decreases as rHDL SPM content increases. The inhibition of LCAT by SPM at the active site has a minimal effect in the modulation of enzyme activity with these substrates. The results of our studies with lipophilic probes suggest that SPM significantly changes the properties of the phospholipid interface at the surface and also in the backbone and acyl chain regions. Furthermore, these changes in the surface properties of the rHDL correlate with decreased LCAT binding and reactivity.

REFERENCES

1. Barenholz, Y., and Gatt, S. (1982) in Phospholipids (Hawthorne, J. N., and Ansell, G. B., ed) pp. 129–177, Elsevier Biomedical Press, Amsterdam
2. Lund-Katz, S., Laboda, H. M., McLean, L. M., and Phillips, M. C. (1988) Biochemistry 27, 3416–3423
3. Slote, J. P., and Bierman, E. L. (1988) Biochim. J. 250, 653–658
4. Glomset, J. (1972) in Blood Lipids and Lipoproteins (Nelson, G., ed) pp. 745–787, Wiley, New York
5. Fielding, C. J., Shore, V. G., and Fielding, P. E. (1972) Biochim. Biophys. Res. Commun. 46, 1493–1498
6. Barter, P. J., Hopkins, G. J., and Gorjatschko, L. (1985) Atherosclerosis 58, 97-107
7. Liu, M., Krul, E. S., and Subbiah, P. V. (1992) J. Biol. Chem. 267, 5139–5147
8. Skipperley, V. (1972) in Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism (Nelson, G. J., ed) pp. 471–583, Wiley Interscience, New York
9. Redd, D., and Sterchi, J. M. (1992) Biochim. Biophys. Acta 1127, 28–32
10. Dory, L., Sloop, C. H., Boquet, L. M., Hamilton, R. L., and Roheim, P. S. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 3489–3492
11. Wong, L., Curtiss, L. K., Huang, J., Mann, C. J., Maldonado B., and Roheim, P. S. (1992) J. Clin. Invest. 90, 2370–2375
12. Pownall, H. Pao, Q., and Massey, J. (1985) J. Biol. Chem. 260, 2146–2152
13. Subbiah, P. V., and Liu, M. (1993) J. Biol. Chem. 268, 20156–20163
14. Matz, C. E., and onas, A. (1982) J. Biol. Chem. 257, 4541–4546
15. Jonas, A., and McHugh, H. T. (1983) J. Biol. Chem. 258, 10335–10340
16. Nichols, A. V., Gong, E. L., Blanche, P. J., Forte, T. M., and Anderson, D. W. (1976) Biochim. Biophys. Acta 446, 226–239
17. Jonas, A., and Matz, C. (1982) Biochemistry 21, 6867–6872
18. Bolin, D. J., and Jonas, A. (1994) J. Biol. Chem. 269, 7429–7434
19. Chen, Y., Tanida, T. Y., and Warner, H. (1995) Anal. Chem. 28, 1756–1758
20. Gwynne, J., Brewer, B. J., and Edelhoch, H. (1974) J. Biol. Chem. 249, 2411–2416
21. Loveny, G. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
22. Jonas, A., Sweeney, S. A., and Herbert, P. N. (1984) J. Biol. Chem. 259, 6369–6375
23. Verger, R., Mieras, M. C. E., and deHaas, G. H. (1973) J. Biol. Chem. 248, 4023–4034
24. Jonas, A., Daehler, J. L., and Wilson, E. R. (1985) J. Biol. Chem. 260, 2757–2762
25. Wald, J., and Goomaraghith, E., De Meutter, J., and Jonas, A. (1990) J. Biol. Chem. 265, 20044–20050
26. Chen, Y., Jiang, J. T., and Martinez, H. M. (1972) Biochemistry 11, 4120–4131
27. Jonas, A., van Edvardtstein, A., Churgay, L., Mantulin, W. M., and Assmann, G. (1993) Biochim. Biophys. Acta 1166, 202–210
28. Bonomo, E. A., and Swain, J. B. (1990) Biochemistry 29, 5094–5103
29. Zorich, N., Keedy, K. E., and Jonas, A. (1987) Biochim. Biophys. Acta 919, 181–189
30. Swainey, J. B. (1983) J. Biol. Chem. 258, 1254–1259
31. Jonas, A., and Jonas, A. (1993) J. Biol. Chem. 268, 4798–4805
32. Calhoun, W. I., and Shlip, G. G. (1979) Biochemistry 18, 1717–1721
33. Weber, G., and Farris, F. (1979) Biochemistry 18, 3075–3078
34. Massey, J. B., She, H. S., and Pownall, H. J. (1985) Biochem. Biophys. Acta 2449, 6973–6978
35. Schmidt, C. F., Barenholz, Y., and Thompson, T. E. (1977) Biochemistry 16, 2649–2656
36. Jonas, A. (1991) Biochim. Biophys. Acta 1084, 205–220
37. Jonas, A., Keedy, K. E., and Wald, J. H. (1989) J. Biol. Chem. 264, 4818–4824
38. Massey, J. B., Pao, Q., Van Winkle, W. B., and Pownall, H. J. (1985) J. Biol. Chem. 260, 11719–11723
39. Sheetz, M. P., and Chan, S. I. (1972) Biochemistry 11, 4573–4581
40. Lichtenberg, D., Freire, E., Schmidt, C. F., Barenholz, Y., Felger, P. L., and Thompson, T. E. (1983) Biochemistry 20, 3462–3467
41. Small, D. M. (1990) in The Physical Chemistry of Lipids, pp. 382–386, 481–515, Plenum Press, New York
42. Barenholz, Y., and Thompson, T. E. (1980) Biochim. Biophys. Acta 604, 129–158
43. Barenholz, Y. (1984) in Physiology of Membrane Fluidity (Shinitzky, M., ed) pp. 131–173, CRC Press, Boca Raton, Fl.
44. Abrahamsson, S., Dahlen, B., Lofgren, H., Pascher, I., and Sundel, S. (1977) in Structure and Biological Membranes (Abrahamsson, S., and Pascher, I., ed) pp. 1–24, Plenum Press, New York
45. Rye, K. A., Hime, N. J., and Barter, P. J. (1996) J. Biol. Chem. 271, 4243–4250