Biochimica et Biophysica Acta, 572 (1979) 452—460

BBA 57337

PHOSPHOLIPASE A_2 IN RAT-LUNG MICROSOMES: SUBSTRATE SPECIFICITY TOWARDS ENDOGENOUS PHOSPHATIDYLCHOLINES

W.J. LONGMORE *, V. OLDENBORG and L.M.G. VAN GOLDE **

Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, 3572 BP Utrecht (The Netherlands)

(Received October 3rd, 1978)

Key words: Phospholipase A_2; Phosphatidylcholine; Substrate specificity; (Rat lung)

Summary

1. Isolated rat lungs were perfused with a variety of radioactive precursors to label the phosphatidylcholines of the microsomal and lamellar body fractions. These endogenously labelled phosphatidylcholines were used as substrates in experiments to identify and characterize phospholipase A activity in lung subcellular fractions.

2. The microsomal fraction was found to contain a phospholipase A specific for the 2-position of endogenous phosphatidylcholines. The enzyme operated optimally at pH 8.5 and required 10 mM Ca^{2+} for maximal activity.

3. No evidence was found for the existence of phospholipase A activity in lamellar bodies.

4. The microsomal phospholipase A_2 was more active towards phosphatidylcholines containing an unsaturated fatty acid at the 2-position than towards the disaturated phosphatidylcholines.

5. Microsomal disaturated phosphatidylcholines labelled with [1-^{14}C]acetate (endogenously synthesized palmitate) were hydrolysed by the microsomal phospholipase A_2; however, no hydrolysis occurred when [9,10-$^{3}H_2$]palmitate was used as a precursor notwithstanding the fact that the label from [1-^{14}C]-acetate is mainly incorporated into the 1-position and that from [9,10-$^{3}H_2$]-palmitate almost exclusively into the 2-position of the disaturated phosphatidylcholines of rat-lung microsomes.

6. These results suggest the existence of two pools of disaturated phosphatidylcholines in rat-lung microsomes. They are consistent with the concept that dipalmitoylphosphatidylcholine synthesized by remodeling of unsaturated

* Present address: Edward A. Doisy Department of Biochemistry, St. Louis University School of Medicine, St. Louis, MO 63104, U.S.A.

** To whom requests for reprints should be addressed.

phosphatidylcholines with exogenously supplied palmitic acid, is not hydrolysed by phospholipase A_2 of lung microsomes.

Introduction

Dipalmitoylphosphatidylcholine is the major surface active component of pulmonary surfactant [1—3]. Both studies in vivo [4,5] and in vitro [6,7] have shown, however, that the principal phosphatidylcholines synthesized de novo are species which contain palmitic acid at the 1-position and an unsaturated fatty acid at the 2-position. Thus, the synthesis of dipalmitoylphosphatidylcholine must be the result of a mechanism which replaces the unsaturated fatty acid at the 2-position with palmitic acid. Three mechanisms have been suggested: (1) a deacylation-reacylation cycle [4,8—10] (b) a deacylation-transacylation process [11,12] and (c) a direct exchange of the unsaturated fatty acid with palmitate [13,14]. Whereas the third process has not yet been thoroughly studied, the first two of these mechanisms clearly require the presence of a phospholipase A_2 (EC 3.1.1.4). A phospholipase A_2 activity has been shown in rat-lung microsomes using exogenously added 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoethanolamine as substrate [15]. However, studies on lung phospholipase A_2 using membrane-bound phosphatidylcholines as substrates and on the substrate specificity towards phosphatidylcholines of different fatty acid composition have not been reported.

It is generally accepted that the type II alveolar epithelial cells are the sole site of synthesis, storage and secretion of the pulmonary surfactant onto the alveolar surface (for recent reviews, see Refs. 2 and 16). The type II alveolar pneumocyte is characterized by the presence of unique organelles called lamellar bodies. It is within these organelles that the surfactant is stored prior to secretion [17]. Evidence has been recently reported for the absence of de novo synthesis of phosphatidylcholine in lamellar bodies [14,18]. These findings endorsed earlier electron microscopic autoradiography studies [19,20] which demonstrated that the bulk of surfactant lipids is synthesized in the endoplasmic reticulum of the type II cell. The possibility remains, however, that the lamellar body may somehow be involved in the remodelling of unsaturated phosphatidylcholines into dipalmitoylphosphatidylcholine. Although lamellar bodies lack the enzymes lysolecithin acyltransferase and lysolecithin : lysolecithin acyltransferase [18,21] which catalyse the acylation of 1-acyl-*sn*-glycero-3-phosphocholine [22] and the transacylation between 2 molecules of 1 acyl-*sn*-glycero-3-phosphocholine [23], respectively, there is evidence for a role of the lamellar bodies in a direct exchange of the unsaturated fatty acid at the 2-position of phosphatidylcholine with palmitate [24]. Conflicting reports as to whether the lamellar body possesses a phospholipase A_2 activity have recently appeared [14,25].

Using isolated microsomal and lamellar body fractions with endogenously labelled phosphatidylcholines as substrate, this communication further characterizes the phospholipase A activity of lung microsomes and establishes the absence of such activity in lamellar bodies.

Materials and Methods

Preparation of endogenously labelled microsomal and lamellar body fractions of rat lung. Rats (Wistar strain), weighing 200—400 g, were obtained from the Central Institute for the Breeding of Laboratory Animals, Zeist, The Netherlands. The rats were anaestetized and the lungs surgically removed and perfused as previously described [26]. The medium used for perfusion was Krebs-Ringer bicarbonate which was equilibrated with the same gas mixture as was used for lung ventilation (90% O_2, 10% CO_2) [27]. Depending upon the label desired, either [*Me*-^{14}C]choline (10 μCi/lung), [1,3-^{3}H]glycerol (0.5 mCi/lung), [1-^{14}C]palmitate (25 μCi/lung), [9,10-^{3}H]palmitate (0.5 mCi/lung), [1-^{14}C]oleate (25 μCi/lung), [1-^{14}C]linoleate (25 μCi/lung), [^{32}P]phosphate (0.5 mCi/lung) or [1-^{14}C]acetate (80 μCi/lung) were added to the perfusion medium. The lungs were perfused for 2 h following the addition of the labelled substrate. After termination of the perfusion, microsomal and lamellar body fractions were isolated as previously described [18]. The microsomal and lamellar body fractions were suspended in 0.25 M sucrose/0.01 M Tris-HCl (pH 7.4)/1 mM EDTA. Following assay of the protein content [28], the subcellular fractions were stored overnight at −80°C prior to use. In each case, the labelling provided approx. 20 000 dpm/0.5 mg microsomal protein and 20 000 dpm/0.2 mg lamellar body protein.

Determination of phospholipase A activity in the microsomal and lamellar body fractions. The subcellular fractions containing endogenously labelled phosphatidylcholines, were incubated at 37°C for up to 2 h in a standard medium containing 50 mM Tris-HCl (pH 8.5), 1 mM EGTA, 10 mM $CaCl_2$ in a total volume of 0.5 ml. Protein concentration was maintained between 0.5 and 0.8 mg/ml for the microsomal fraction and between 0.1 and 0.3 mg/ml for the lamellar body fraction. The reactions were started by the addition of the Ca^{2+} and terminated by adding 2 ml $CH_3OH/CHCl_3$ (2 : 1, v/v). After extraction of the lipids via the procedure of Bligh and Dyer [29], phosphatidylcholine, lysophosphatidylcholine and, in some experiments, the free fatty acids fraction were isolated via thin-layer chromatography and assayed for radioactivity by means of liquid-scintillation counting [7].

In a number of experiments, the isolated phosphatidylcholines were divided into aliquots so that a portion could be used for assay of the radioactivity in the various molecular classes of phosphatidylcholine. This was accomplished by argentation thin-layer chromatography after conversion of the phosphatidylcholines either into 1,2-diacyl-*sn*-glycerols [30] or into dimethylphosphatidates [31].

Other procedures. Disaturated phosphatidylcholines were isolated from the total phosphatidylcholine fraction by the method described by Mason et al. [32]. The positional distribution of radioactive fatty acids among the 1- and 2-position of disaturated and total phosphatidylcholines was determined by means of degradation with phospholipase A_2 from snake venom [30]. Separation of radioactive palmitate from other labelled fatty acids synthesized during perfusion of the lung with [1-^{14}C]acetate, was accomplished by argentation chromatography of the methylester-derivatives of the fatty acids [33].

Materials. All radiochemicals were purchased from the Radiochemical

Centre, Amersham, U.K. Pure phospholipase A_2 from *Naja naja* venom was a generous gift of Dr. W. Renooij (Department of Veterinary Biochemistry, Utrecht). Other biochemicals were obtained from Boehringer, Mannheim, F.R.G.

Results

Optimal conditions for assay of phospholipase A in lung microsomes

Optimal conditions of pH, Ca^{2+} and protein concentration were determined in microsomes isolated from lungs perfused with [*Me*-^{14}C]choline. The pH optimum was found to be pH 8.5. Activity at pH 7.5 and 9.0 was 42% and 73%, respectively, of that measured at pH 8.5. The activity was found to be stimulated by Ca^{2+} with a maximum activation at 10 mM Ca^{2+}. Without added Ca^{2+} and in the presence of 1 mM EGTA, the activity was only 14% of that measured with 10 mM Ca^{2+} present.

Positional specificity of lung microsomal phospholipase

To determine whether the phospholipase activity of lung microsomes was of the A_1 or A_2 classification and to investigate the specificity towards saturated or unsaturated fatty acyl moieties, microsomal phosphatidylcholine was endogenously labelled by lung perfusion with [1-^{14}C]palmitate, [1-^{14}C]oleate or [1-^{14}C]linoleate. Following incubation of the isolated endogenously labelled microsomes in the presence of Ca^{2+} for 0, 60 and 120 min, the radioactivity at the 1- and 2-position of the remaining phosphatidylcholines was determined by means of degradation with pure phospholipase A_2 from *Naja naja* venom (Table I). Phosphatidylcholines from microsomes endogenously labelled with [1-^{14}C]oleate or [1-^{14}C]linoleate were hydrolysed to an extent of 17 and 16% per h respectively. This loss was paralleled by the decrease in radioactivity at the 2-position of phosphatidylcholine, whereas no loss occurred in the radioactivity at the 1-position. These results clearly indicate that the phospholipase A of lung microsomes is of the A_2 classification with no detectable A_1 activity. The data presented in Table I demonstrate that microsomal phosphatidylcholine labelled by lung perfusion with [1-^{14}C]palmitic acid is not hydrolysed at all. At first view, this would suggest that the microsomal phospholipase A_2 requires the presence of an unsaturated fatty acid at the 2-position of phosphatidylcholine.

Specificity of phospholipase A_2 towards different molecular classes of endogenously labelled phosphatidylcholines

Microsomes were isolated from lungs perfused with either [1,3-^{3}H]glycerol, [^{32}P]phosphate or [1-^{14}C]acetate. In each case, the labelled microsomes were incubated under standard conditions for 0, 60 and 120 min. Following incubation, 1,2-diacyl-*sn*-glycerols or dimethylphosphatidates derived from the remaining phosphatidylcholines were separated by argentation thin-layer chromatography in order to establish the degree of degradation of the disaturated, mono- and dienoic classes of phosphatidylcholine. The tetraenoic class was not sufficiently labelled to permit accurate analysis. The results (Table II) demonstrate that the radioactivity decreased the least in the disaturated class,

TABLE I

PHOSPHOLIPASE A_2 ACTIVITY OF RAT-LUNG MICROSOMES USING ENDOGENOUSLY LABELLED PHOSPHATIDYLCHOLINES AS SUBSTRATE

Microsomes were isolated from lungs perfused for 2 h with 25 μCi of the specified fatty acid. Subsequently, the microsomes were incubated in the presence of Ca^{2+}. Before and after the incubation, aliquots of the phosphatidylcholines were treated with a pure phospholipase A_2 from *Naja naja* venom to determine the radioactivity at the 1- and 2-position of phosphatidylcholine. The results are expressed as percentage decrease of radioactivity in total phosphatidylcholine, at the 1- and at the 2-position of phosphatidylcholine. At zero incubation time, the 2-position of phosphatidylcholine contained 92% of the label of [1-^{14}C]linoleic acid incorporated into this phospholipid, 90% of the label of [1-^{14}C]oleic acid and 69% of the label of [1-^{14}C]palmitic acid. Averages (± S.D.) of three incubations of at least duplicate experiments have been presented.

Endogenous label	Decrease of radioactivity (% per h)		
	Total phosphatidylcholine	1-position	2-position
[1-^{14}C]Linoleic acid	16 ± 2	n.d. *	15 ± 1
[1-^{14}C]Oleic acid	16 ± 1	n.d.	18 ± 2
[1-^{14}C]Palmitic acid	n.d.	n.d.	n.d.

* n.d., no detectable decrease.

averaging a 13% loss after 60 min of incubation. The loss of radioactivity of the mono- and dienoic phosphatidylcholines, however, was found to average 29 and 27%, respectively, per 60 min. These results indicate that the phospholipase A_2 is significantly more active towards phosphatidylcholines containing an unsaturated fatty acid at the 2-position as compared to the disaturated species of phosphatidylcholine.

Differential specificity of microsomal phospholipase A_2 towards de novo synthesized and exogenously supplied palmitate of phosphatidylcholine

It is clear from the data presented in Table I and II that the activity of the microsomal phospholipase A_2 towards the disaturated species of phosphatidyl-

TABLE II

SPECIFICITY OF PHOSPHOLIPASE A_2 OF RAT-LUNG MICROSOMES FOR VARIOUS CLASSES OF ENDOGENOUSLY LABELLED PHOSPHATIDYLCHOLINES

Microsomes were isolated from lungs perfused for 2 h with either 0.5 mCi [1,3-^{3}H]glycerol, 0.5 mCi [^{32}P]phosphate or 80 μCi [1-^{14}C]acetate. Subsequently, the microsomes were isolated and incubated in the presence of Ca^{2+} for up to 2 h. Before and after incubation, the disaturated, monoenoic and dienoic fractions of phosphatidylcholine were resolved and assayed for radioactivity. The results are expressed as decrease (% per h) of total radioactivity in the various molecular classes of phosphatidylcholine. Averages (± S.D.) of three incubations are presented.

Endogenous label	Decrease of radioactivity (% per h)		
	Disaturated PC	Monoenoic PC	Dienoic PC
[1,3-^{3}H]Glycerol	19 ± 2	33 ± 4	26 ± 2
[^{32}P]Phosphate	10 ± 1	26 ± 3	18 ± 2
[1-^{14}C]Acetate	9 ± 1	28 ± 3	38 ± 4

choline containing de novo synthesized palmitate differed markedly from the activity towards disaturated phosphatidylcholines labelled with exogenously supplied palmitic acid. The former were cleaved at a similar rate (9% per h) as disaturated phosphatidylcholines labelled with [^{32}P]phosphate or [1,3-^{3}H]-glycerol (Table II). No detectable cleavage could, however, be measured when phosphatidylcholine was labelled with exogenously supplied palmitate (Table I). An intriguing possibility to explain these different activities of the microsomal phospholipase A_2 towards phosphatidylcholines containing palmitate of different origin, is that in lung microsomes two pools of disaturated phosphatidylcholines exist which can be distinguished by the enzyme. One pool would be a disaturated species containing endogenously synthesized palmitate in the 2-position, whereas the other pool contained exogenously supplied palmitate in the 2-position.

In order to establish whether indeed two different pools of disaturated phosphatidylcholines exist, lungs were perfused with either [1-^{14}C]acetate or [9,10-$^{3}H_2$]palmitate followed by isolation of the microsomal phosphatidylcholines. Table III shows the positional distribution of [^{14}C]palmitate, synthesized de novo from [1-^{14}C]acetate, and that of exogenously supplied [^{3}H]palmitate between the 1- and 2-position of total microsomal phosphatidylcholines and disaturated phosphatidylcholines. It is interesting to note that the palmitate synthesized de novo is recovered predominantly at the 1-position of total phosphatidylcholines and disaturated phosphatidylcholines. In strong contrast, the exogenously added palmitate incorporated preferentially into the 2-position of total phosphatidylcholines and, even more pronounced, into the 2-position of disaturated phosphatidylcholines. In this light it is relevant to mention that the majority of the 2-position palmitate in total phosphatidylcholine resides in the disaturated species of this phospholipid. These data strongly suggest the presence of two pools of disaturated phosphatidylcholines in lung microsomes.

Examination of lamellar bodies for phospholipase A activity

Lamellar bodies were isolated from lungs perfused with either [*Me*-^{14}C]-choline or [1,3-^{3}H]glycerol. Subsequently, the lamellar bodies were incubated for periods up to 2 h followed by isolation of lysophosphatidylcholine and phosphatidylcholine to test for phospholipase A activity. The basic incubation conditions were similar to those used for the microsomal phospholipase A assay. No phospholipase activity was detected in lamellar bodies at pH 4.0, 7.5 or 8.5 in the presence or absence of 0.01% (w/v) Triton X-100 or when the lamellar body preparation was sonicated prior to incubation (data not shown).

Discussion

While earlier studies had suggested a phospholipase A activity in lung tissue [34], phospholipase A_2 activity in the lung was first demonstrated by Ohta et al. [35]. They found, using both phosphatidylcholine and phosphatidylethanolamine as exogenous substrates, the majority of the phospholipase A_2 activity in the 105 000 $\times g$ fraction of lung homogenate. Surprisingly, the activity was not stimulated by Ca^{2+} [35]. More recently, Garcia et al. [15] described a phospho-

lipase A_2 activity in rat lung microsomal and mitochondrial fractions. This activity, which required Ca^{2+}, was determined using exogenously added phosphatidylethanolamine as substrate. In the present study, it was desired to study the activity of phospholipase A_2 in lung microsomes using membrane-bound phosphatidylcholines, generated by perfusion of the lung with various radioactive precursors, as substrate. This was necessary, as exogenously added radioactive phosphatidylcholine failed to demonstrate the presence of significant phospholipase A activity in lung microsomes. In addition, it has been previously shown for other enzymes associated with phospholipid metabolism [36] that membrane-bound substrates rather than emulsions of exogenously added substrates should be used to investigate the substrate specificity of these enzymes.

The microsomal phospholipase A_2 described in the present study possessed a similar pH optimum and Ca^{2+}-requirement as that reported by Garcia et al. [15].

All attempts to demonstrate phospholipase A activity in the lamellar body fraction were unsuccessful. This finding agrees with that of Garcia et al. [14] but is in conflict with the report of Heath and Jacobson [25]. As the preparation of lamellar bodies in the latter report was considered only a lamellar body-rich fraction, the reported activity could be due to contamination with other subcellular fractions.

Importantly, the present study demonstrates that the microsomal phospholipase A_2 is more active towards phosphatidylcholines containing an unsaturated fatty acid at the 2-position. This observation endorses the potential significance of phospholipase A_2 in the remodelling of phosphatidylcholines synthesized de novo, because previous studies have shown that the synthesis de novo results primarily in the formation of phosphatidylcholines containing an unsaturated fatty acid at the 2-position [4—7].

Complicating this study was the observation that there was no demonstrable phospholipase A_2 activity if the microsomal phosphatidylcholine had been labelled by perfusing the lung with trace amounts of [1-^{14}C]palmitic acid (Table I), although this fatty acid is recovered almost completely at the 2-position of disaturated phosphatidylcholine (Table III). However, the disaturated class of phosphatidylcholine labelled endogenously with palmitate synthesized during perfusion with trace amounts of [1-^{14}C]acetate, was cleaved by the phospholipase A_2 although at half the rate of phosphatidylcholines containing an unsaturated fatty acid at the 2-position. In conjunction with the strikingly different positional distribution of de novo synthesized and exogenously added palmitate among the 1- and 2-position of disaturated phosphatidylcholines (Table III), these findings suggest the presence of two pools of disaturated phosphatidylcholines: one pool containing endogenously synthesized palmitic acid at the 2-position which is accessible for the phospholipase A_2 and the other pool containing exogenously supplied palmitate at the 2-position which is not accessible for cleavage with phospholipase A_2. It is interesting to speculate that the latter pool may represent phosphatidylcholines which have been remodelled to dipalmitoylphosphatidylcholine for surfactant production which should be inaccessable to the phospholipase. Such a situation would allow for the accumulation of dipalmitoylphosphatidylcholine and its eventual storage in

TABLE III

POSITIONAL DISTRIBUTION OF DE NOVO SYNTHESIZED AND EXOGENOUSLY SUPPLIED PALMITIC ACID BETWEEN THE 1- AND 2-POSITION OF TOTAL PHOSPHATIDYLCHOLINES AND DISATURATED PHOSPHATIDYLCHOLINES OF RAT-LUNG MICROSOMES

Lungs were perfused for 2 h with 50 μCi [1-^{14}C]acetate or 0.5 mCi [9,10-$^{3}H_2$]palmitic acid. Subsequently, the microsomal total phosphatidylcholine fraction and the disaturated class of phosphatidylcholine were isolated and subjected to hydrolysis with pure phospholipase A_2 from *Naja naja* venom to ascertain the positional distribution (%) of the endogenously synthesized palmitate and the exogenously supplied palmitate. Averages of two experiments are presented.

Source of palmitic acid	Total phosphatidylcholines		Disaturated phosphatidylcholines	
	1-position	2-position	1-position	2-position
Synthesized from [1-^{14}C]acetate	74	26	71	29
Exogenously supplied as [9,10-$^{3}H_2$]palmitic acid	33	67	8	92

the lamellar body. If this hypothesis were true, it would imply that palmitic acid synthesized de novo would be used primarily for the synthesis of phosphatidylcholines de novo whereas palmitic acid taken up from the circulation would be used primarily for the remodelling of unsaturated phosphatidylcholines into dipalmitoylphosphatidylcholine. It should be emphasized, however, that the present studies have been carried out with microsomes isolated from whole lung and that additional studies with microsomes from alveolar type II cells are required to further investigate this hypothesis.

Acknowledgements

The investigations were supported in part by the Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), and by the U.S. Public Health Service through a Fogarty International Senior Research Fellowship (No. Fo5-TW00099) and Grant No. HL-13405 to W.J.L.

References

1 Goerke, J. (1974) Biochim. Biophys. Acta 344, 241—261
2 Frosolono, M.F. (1977) in Lipid Metabolism in Mammals (Snyder, F., ed.), Vol. 2, pp. 1—38, Plenum Press, New York
3 King, R.J. and Clements, J.A. (1972) Am. J. Physiol. 223, 715—726
4 Vereyken, J.M., Montfoort, A. and van Golde, L.M.G. (1972) Biochim. Biophys. Acta 260, 70—81
5 Moriya, T. and Kanoh, H. (1974) Tohoku J. Exp. Med. 112, 241—256
6 Akino, T., Abe, M. and Arai, T. (1971) Biochim. Biophys. Acta 248, 274—281
7 Sarzala, M.G. and van Golde, L.M.G. (1976) Biochim. Biophys. Acta 441, 423—432
8 Frosolono, M.F., Slivka, S. and Charms, B.L. (1971) J. Lipid Res. 12, 96—103
9 Snyder, F. and Malone, B. (1975) Biochem. Biophys. Res. Commun. 66, 914—919
10 Tansey, F.A. and Frosolono, M.F. (1975) Biochem. Biophys. Res. Commun. 67, 1560—1566
11 Akino, T., Yamazaki, I. and Abe, M. (1972) Tohoku J. Exp. Med. 108, 133—139
12 Hallman, M. and Raivio, K. (1974) Pediat. Res. 8, 874—879
13 Kyei-Aboagye, K., Rubinstein, D. and Beck, J.C. (1973) Can. J. Biochem. 51, 1581—1587
14 Garcia, A., Sener, S.F. and Mavis, R.D. (1976) Lipids 11, 109—112
15 Garcia, A., Newkirk, J.D. and Mavis, R.D. (1975) Biochem. Biophys. Res. Commun. 64, 128—135
16 Van Golde, L.M.G. (1976) Am. Rev. Respir. Dis. 114, 977—1000

17 Askin, F.B. and Kuhn, C. (1971) Lab. Invest. 25, 260—268
18 Baraňska, J. and van Golde, L.M.G. (1977) Biochim. Biophys. Acta 488, 285—293
19 Chevalier, G. and Collet, A.J. (1972) Anat. Rec. 174, 289—310
20 Petrik, P. and Collet, A.J. (1974) Am. J. Anat. 139, 519—533
21 Tsao, F.H.C. and Zachman, R.D. (1977) Pediat. Res. 11, 849—857
22 Lands, W.E.M. (1960) J. Biol. Chem. 235, 2233—2237
23 Erbland, J.F. and Marinetti, G.V. (1965) Biochim. Biophys. Acta 105, 128—138
24 Engle, M.J., Sanders, R.L. and Longmore, W.J. (1976) Arch. Biochem. Biophys. 173, 586—595
25 Heath, M.F. and Jacobson, W. (1976) Biochim. Biophys. Acta 441, 443—452
26 Godinez, R.I. and Longmore, W.J. (1973) J. Lipid Res. 14, 138—144
27 Moxley, M.A. and Longmore, W.J. (1975) Life Sci. 17, 921—925
28 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
29 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911—917
30 Van Golde, L.M.G. and van Deenen, L.L.M. (1966) Biochim. Biophys. Acta 125, 496—509
31 Renkonen, O. (1968) Biochim. Biophys. Acta 152, 114—135
32 Mason, R.J., Nellenbogen, J. and Clements, J.A. (1976) J. Lipid Res. 17, 281—284
33 Lees, A.M. and Korn, E.D. (1966) Biochim. Biophys. Acta 116, 403—406
34 Gallai-Hatchard, J.J. and Thompson, R.H. (1965) Biochim. Biophys. Acta 98, 128—136
35 Ohta, M. and Hasegawa, H. (1972) Tohoku J. Exp. Med. 108, 85—94
36 Kanoh, H. and Ohno, K. (1975) Biochim. Biophys. Acta 380, 199—207