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Study of properties of cholinephosphotransferase from fetal guinea pig lung mitochondria and microsomes*

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Summary

We have reported earlier that cholinephosphotransferase (EC 2.7.8.2) is present in both mitochondria and microsomes of fetal guinea pig lung. This study was designed to compare the properties of mitochondrial and microsomal cholinephosphotransferase in fetal guinea pig lung. Various parameters, such as substrate specificity, K_m values, sensitivity to N-ethylmaleimide, dithiothreitol and trypsin were measured. Both showed significant preference for unsaturated diacylglycerols over saturated diacylglycerols. Data on K_m and V_{max} indicate that the affinity of this enzyme for different diacylglycerols varies between the two forms. The ID_{50} values for N-ethylmaleimide were 20 mM and 12.5 mM for the mitochondrial and microsomal form of the enzyme, respectively. Dithiothreitol showed an inhibitory effect on both; however, the mitochondrial form was inhibited less than the microsomal form. The effects of N-ethylmaleimide and dithiothreitol on both forms of enzyme indicated that the microsomal cholinephosphotransferase requires a higher concentration of -SH for its activity than the mitochondrial enzyme does. The enzyme was inhibited by trypsin in both mitochondria and microsome under isotonic condition suggesting that this enzyme is on the outside of the membrane in both endoplasmic reticulum and mitochondria.

Introduction

The lung becomes functional at birth, when it undergoes a transition from a liquid to gas filled environment. This transition can not occur successfully in the absence of a surfactant system in alveolar type II cells. Pulmonary surfactant is a lipoprotein in which the lipid fraction consists predominantly of phosphatidylcholine, over 60% of which is saturated [1]. This saturated phosphatidylcholine is largely dipalmitoylphosphatidylcholine [2, 3]. Many investigators have studied the pathways involved in the formation of pulmonary lipids, with particular emphasis on the biosynthesis of dipalmitoylphosphatidylcholine [4]. It is now generally believed that surfactant dipalmitoylphosphatidylcholine can be directly synthesized *de novo* via CDP-choline pathway [5–9] or by a concerted action between synthesis *de novo* and remodelling of unsaturated phosphatidyl choline [10].

Even though cholinephosphocytidyltransferase is known to catalyse the rate limiting step in the CDP-choline pathway in type II cells [11] and other cells [12] in providing CDP-choline, cholinephosphotransferase plays also a regulatory role since it

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does exhibit specificity towards endogenous diacylglycerol species [4, 13].

It is generally believed that cholinephosphotransferase is exclusively present in endoplasmic reticulum [14]. However, we have reported the presence of a mitochondrial form of this enzyme in fetal and adult guinea pig lung and liver [15–18] and adult guinea pig alveolar type II cells [19]. The activity in fetal lung mitochondria was more than twice that in fetal microsome [15]. Extramicrosomal presence of cholinephosphotransferase has also been reported by others [20–23]. Although microsomal cholinephosphotransferase has been shown to have a general lack of selectivity towards various molecular species if diacylglycerol in rat lung microsome [6, 7] and in type II cells [8, 11], there are some studies in which it exhibits a preference for unsaturated diacylglycerols [13, 24–26]. To our knowledge, no one has studied whether there is any similarity or difference in the kinetic properties of the mitochondrial and microsomal cholinephosphotransferase. The purpose of this study was to compare the properties of microsomal and mitochondrial cholinephosphotransferase in fetal guinea pig lung.

Experimental procedure

Animals

Pregnant guinea pigs of known gestational ages were obtained from Camm Laboratory Animals, Wayne, New Jersy. All animals were housed in stainless steel cages and fed *ad libitum*. Fresh lettuce was provided once a week as an additional source of vitamin C.

Chemicals

CDP- (methyl-^{14}C) choline (spec. act. 52.5 Ci/mol) was purchased from New England Nuclear Corporation, Boston, MA. 1,2-diacylglycerols (free from oxidized fatty acids and 1,3-isomer of diacylglycerols) were obtained from Nu Check Prep., Elysean, MN and Serdery Research Labs., Ontario, Canada. Other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo. Butanol was purchased from Malinckrodt Inc, Paris, KY. Universol cocktail was purchased from ICN radiochemicals, Irvine, CA.

Subcellular preparations

At 55 days of gestation, the fetuses were delivered by Cesarean section and the lungs from these fetuses were removed. Lungs were washed in ice cold 0.9% saline, weighed and homogenized in two volumes of 0.25 M sucrose/1 mM EDTA (pH 7.4) with a Potter Elvehjem homogenizer. The nuclear fraction was prepared by centrifuging the whole homogenate at 600 × g for 10 min in a refrigerated Sorvall RC-5 centrifuge using a SS-34 rotor. The nuclear pellet was washed once with 0.25 M sucrose/1 mM EDTA (pH 7.4). The supernatants were combined and centrifuged at 10,000 × g for 10 min to obtain the mitochondrial fraction. The supernatant was centrifuged in a Beckman L8-55M ultracentrifuge at 105,000 × g for 60 min using a 70.1 Ti rotor to obtain the microsomal and cytosolic fractions.

The crude mitochondrial fraction was purified by linear sucrose density gradient [17]. About 6.0 ml of 35 to 50% (w/v in 0.1 M Tris/1 mM EDTA, pH 7.4) linear sucrose gradient was made with a Buchler Polystatic Pump (Buchler Instruments) and 2.0 ml of crude mitochondrial fraction was layered on top of the gradient and centrifuged with a Beckman L8-55M ultracentrifuge at 50,000 × g for 60 min in a SW 40 rotor. The pellet obtained was resuspended in Tris HCl/EDTA buffer and centrifuged at 10,000 × g for 10 min. The resulting pellet and other particulate fractions were washed twice successively with 1.15% KCl and 0.1 M Tris HCl/1 mM EDTA, pH 7.4 and suspended in a known volume of buffer. The protein content was determined by the method of Lowry *et al.* [27].

Biochemical assays

Cholinephosphotransferase activity was measured by the incorporation of CDP-(methyl-^{14}C) choline into phosphatidylcholine in freshly prepared mitochondrial and microsomal fractions. The assay was performed as described earlier [15]. All assay contained 1,2-dioleoyl-sn-glycerol as substrates (unless otherwise stated). Mitochondrial and microsomal fractions were checked for cross contam-

ination by assay for marker enzymes, succinate INT-reductase [26] for mitochondria and NADPH cytochrome c reductase [28] for microsome.

For studying the *in vitro* effects of trypsin, N-ethylmaleimide (NEM) and dithiothreitol (DTT) on the activity of cholinephosphotransferase, these reagents were added to the assay mixture as described in individual experiments. Adenylate kinase, a marker of the inner surface of the mitochondrial outer membrane [29] was assayed as described earlier [29].

Statistical analysis

Data were treated statistically using the student's 't' test [30]. The variability of data is presented as mean ± S.E.M. Differences at $p < 0.05$ were considered significant.

Results

Cholinephosphotransferase activity was found in all subcellular fractions as shown in Table 1. The total recovery of protein, activity of cholinephosphotransferase, NADPH cytochrome c reductase and succinic INT-reductase in the subcellular fractions of whole lung homogenate was 88%, 82%, 100.7% and 77.8%, respectively. The total cholinephosphotransferase activity was 3.2-fold higher in the mitochondrial fraction than that in the microsomal fraction. Succinic INT-reductase was distributed in the mitochondria almost 15-fold more than in the microsomal fraction. NADPH cytochrome c reductase showed higher activity in the microsomal fraction (approximately 3.9-fold) as compared with the mitochondrial activity.

The apparent occurence of some NADPH cytochrome c reductase in mitochondria may be explained by the presence of pyridine nucleotide transhydrogenase which converts NADPH to NADH [28]. The NADH formed is utilized by NADH cytochrome c reductase to reduce cytochrome c. Furthermore, the ratio of relative specific activity of cholinephosphotransferase to that of succinic INT-reductase is higher than the ratio of relative specific activity of NADPH cytochrome c reductase to that of succinic INT-reductase in mitochondria, suggesting a true cholinephosphotransferase activity in mitochondria. The presence of NADPH cytochrome c reductase activity in the mitochondrial fraction does not necessarily indicate the presence of microsomal vesicle in this fraction. In fact, ultrastructural examination of mitochondria did not reveal any microsomal contamination (photograph not shown).

The activity of cholinephosphotransferase from

Table 1. Subcellular distribution of cholinephosphotransferase and marker enzyme activity in 55 day old fetal guinea pig lung

Fractions	Cholinephosphotransferase		NADPH-cytochrome c-reductase		Succinic INT reductase	
	Specific	Total	Specific	Total	Specific	Total
Homogenate	0.23	17.6	13.1	1037	8.6	625
	(0.04)	(2.7)	(2.8)	(130)	(0.5)	(43)
Nucleus	0.09	2.9	12.4	377	3.8	108
	(0.02)	(0.9)	(2.4)	(48)	(0.7)	(12.0)
Mitochondria	1.42	6.8	19.4	82	49	287
	(0.26)	(0.9)	(1.7)	(9.4)	(1.5)	(34)
Microsome	0.35	2.1	45	319	4.9	19.5
	(0.06)	(0.5)	(4.7)	(41)	(1.0)	(3.5)
Cytosol	0.13	2.7	10.3	268	2.9	72
	(0.03)	(0.8)	(1.1)	(28)	(0.6)	(13.6)

The specific activity is expressed as nmoles/min/mg of protein. The total activity is expressed as nmoles/gm of tissue. Each value represents the mean ± S.E.M. of five experiments. Cholinephosphotransferase activity was assayed in 50 mM Tris-HCl buffer (pH 8.5) using 20 μg protein from each fraction. Marker enzyme activity was determined in 150 mM potassium phosphate buffer (pH 7.4) using 100 μg of protein

fetal guinea pig lung mitochondria and microsome was linear with time for at least 20 min. The reaction was optimal at pH 8.5 for both the mitochondrial and microsomal forms of the enzyme. Similar observations have also been made for cholinephosphotransferase from adult guinea pig lung mitochondria and microsome [15]. Both mitochondrial and microsomal cholinephosphotransferase showed maximal activity when measured at a concentration of 6 mM for all diacylglycerols.

Table 2 shows the data on the specific activity of mitochondrial and microsomal form of enzyme using different diacylglycerol as substrates. The enzyme from both source showed a preference for unsaturated diacylglycerols over mixed and saturated diacylglycerols. There was some difference between the two forms of enzyme regarding the order of preference. The mitochondrial form of cholinephosphotransferase showed maximum preference for 1,2-dilinolenoyl-sn-glycerol followed by 1,2-dioleoyl-sn-glycerol, 1,2-dilinoleoyl-sn-glycerol, 1-palmitoyl, 2-oleoyl-sn-glycerol, 1-oleoyl, 2-palmitoyl-sn-glycerol, 1-stearoyl-2-linoleoyl-sn-glycerol, 1,2-dimyristoyl-sn-glycerol, 1,2-distearoyl-sn-glycerol and 1,2-dipalmitoyl-sn-glycerol. But in microsome the order of substrate preference varied from mitochondria and was as follows: 1,2-dilinolenoyl-sn-glycerol > 1,2-dioleoyl-sn-glycerol > 1,2-dilinoleoyl-sn-glycerol = 1-palmitoyl-2-oleoyl-sn-glycerol > 1-oleoyl-2-palmitoyl-sn-glycerol > 1-stearoyl-2-linoleoyl-sn-glycerol > 1,2-dipalmitoyl-sn-glycerol > 1,2-distearoyl-sn-glycerol > 1,2-dimyristoyl-sn-glycerol.

In mitochondria, there was no significant difference in cholinephosphotransferase activity between 1,2-dioleoyl-sn-glycerol and 1,2-dilinolenoyl-sn-glycerol as substrate. Among the unsaturated diacylglycerols even though the mitochondria showed some preference towards 1,2-dilinolenoyl-sn-glycerol and 1,2-dioleoyl-sn-glycerol over 1,2-dilinoleoyl-sn-glycerol, in microsome there was no significant difference between these three substrates. All mixed diacylglycerol were used without significant difference by mitochondria, however in microsome significant difference in activity was found between 1-palmitoyl-2-oleoyl-sn-glycerol and 1-stearoyl-2-linoleoyl-sn-glycerol, the former substrate was utilized equally as unsaturated diacylglycerols. Saturated diacylglycerols were utilized without significant difference by the two forms of enzyme. Unsaturated diacylglycerols were used with significant preference when com-

Table 2. The effect of different diacylglycerols on the activity of cholinephosphotransferase from 55 day old fetal guinea pig lung

Substrate	Specific activity	
	Mitochondria	Microsomes
Unsaturated Diacylglycerol		
1,2-dilinolenoyl-sn-glycerol	1.98 ± 0.23	0.59 ± 0.09
1,2-dioleoyl-sn-glycerol	1.53 ± 0.12	0.44 ± 0.02
1,2-dilinoleoyl-sn-glycerol	1.34 ± 0.12[a]	0.36 ± 0.07
Mixed Diacylglycerol		
1-palmitoyl, 2-oleoyl-sn-glycerol	1.19 ± 0.23[a]	0.37 ± 0.05
1-oleoyl, 2-palmitoyl-sn-glycerol	0.87 ± 0.11[d]	0.24 ± 0.03[c]
1-stearoyl, 2-linoleoyl-sn-glycerol	0.76 ± 0.07[d]	0.19 ± 0.03[c]
Saturated Diacylglycerol		
1,2-dimyristoyl-sn-glycerol	0.48 ± 0.16[d]	0.12 ± 0.02[d]
1,2-distearoyl-sn-glycerol	0.39 ± 0.07[d]	0.15 ± 0.05[d]
1,2-dipalmitoyl-sn-glycerol	0.31 ± 0.05[d]	0.17 ± 0.02[d]

These reactions were carried in the presence of 6 mM concentration of the individual diacylglycerols and the values are represented as mean ± S.E.M. of five experiments. The specific activity is expressed as nmoles/min/mg of protein. The activity of cholinephosphotransferase in mitochondria was significantly different between 1,2-dilinolenoyl-sn-glycerol and other substrates (for [a], $p = < 0.05$, for [d], $p = < 0.001$). Activity of cholinephosphotransferase in microsomes was significantly different between 1,2-dilinolenoyl-sn-glycerol and other substrates (for [c], $p = < 0.01$, for [d], $p = < 0.001$).

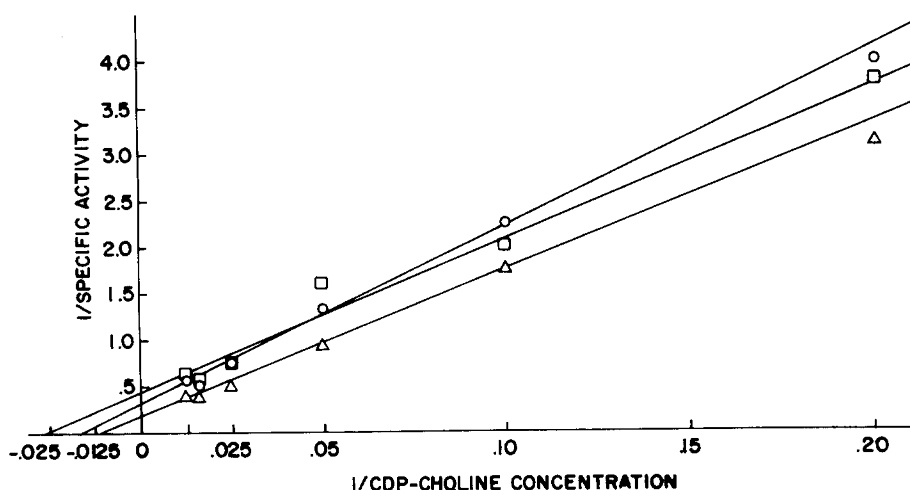

Fig. 1. The activity of cholinephosphotransferase as a function of CDP-choline concentration in the mitochondria of 55-day old fetal guinea pig lung. 20 μg of protein were used in each assay. Each point represents the mean of four experiments. 1,2-dilinolenoyl-sn-glycerol (△—△), 1,2-dioleoyl-sn-glycerol (O—O) and 1-palmitoyl-2-oleoyl-sn-glycerol (□—□).

pared to saturated diacylglycerols in both mitochondria and microsome ($p = < 0.001$), only 1,2-dilinoleoyl-sn-glycerol showed a lower level of significance with saturated diacylglycerol (p between 0.05 and 0.01) in microsome.

The effect of CDP-choline concentration, using different diacylglycerols as substrates on the enzyme activity in mitochondria and microsome is shown in Figs 1 and 2. Maximal enzyme activity was observed at 60 μM and 80 μM of CDP-choline concentration for mitochondrial and microsomal forms, respectively. In mitochondria the apparent K_m values for CDP-choline at a diacylglycerol concentration of 6 mM were 59.05 ± 1.22, 87.51 ± 5.60 and 37.63 ± 1.05 for 1,2-dioleoyl-sn-glycerol, 1,2-dilinolenoyl-sn-glycerol and 1-palmitoyl-2-oleoyl-sn-glycerol as substrates, respectively. V_{max} values were 3.01, 5.22 and 2.06 n mole/min/mg of protein for the respective substrates. For microsomal enzyme, the apparent K_m values were 65.00 ± 8.60, 28.80 ± 3.70 and 42.45 ± 8.48 respectively for the previously listed diacylglycerols. V_{max} values were 1.11, 1.25 and 0.75 n mole/min/mg of protein for the same order of substrates.

Trypsin is a protease which catalyses the hydrolysis of peptide bond whose carbonyl function is donated by a lysine and an arginine residue, had inhibitory effect on cholinephosphotransferase as shown in Table 3. Under isotonic condition, both mitochondrial and microsomal form of enzyme showed 66.8% and 70.9% inhibition, respectively, at 40 μM trypsin concentration during incubation. Trypsin treatment under hypotonic condition had almost similar inhibitory effect on both form of enzyme. We also studied the effect of trypsin on the enzyme activity suspending the mitochondrial and microsomal pellet in either isotonic medium (0.25 M sucrose, 5 mM potassium phosphate, 0.1 mM EDTA, 1 mM dithiothreitol, pH 7.5) or hypotonic medium (0.05 M sucrose, 5 mM potassium phosphate, 0.1 mM EDTA, 1 mM dithiothreitol, pH 7.5). The mitochondrial and microsomal fractions were incubated at 37° C for 5 min with trypsin (5.0 mg/ml in respective buffer at pH 7.5). The protein : trypsin ratio was 1 : 2; after 5 min of incubation, an excess of soybean trypsin inhibitor (inhibitor : protein, 6 : 1 w/w) was added to both control and trypsin treated samples. The samples with or without trypsin were assayed for cholinephosphotransferase activity. There was about 92 to 96% inhibition for both forms of enzyme under both isotonic and hypotonic conditions, suggesting that both enzymes are on outer side of the membrane. Furthermore, the presence of cholinephosphotransferase in the inner side of the outer membrane of mitochondria can be ruled out because trypsin

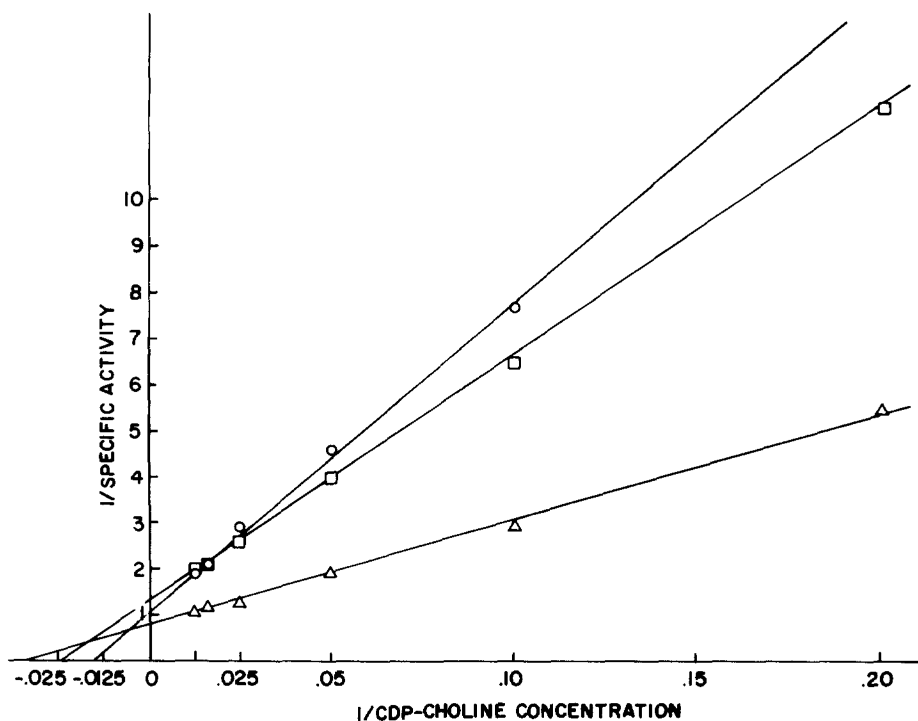

Fig. 2. The activity of cholinephosphotransferase as a function of CDP-choline concentration in the microsomes of fetal guinea pig lung. 20 μg of protein were used in each assay. Each point represents the mean of four experiments. 1,2-dilinolenoyl-sn-glycerol (△—△), 1,2-dioleoyl-sn-glycerol (○—○) and 1-palmitoyl-2-oleoyl-sn-glycerol (□—□).

inhibited the activity of adenylate kinase, a marker for the inner surface of the outer mitochondrial membrane, only under the hypotonic condition and not under the isotonic condition.

Effect of exposure of the mitochondrial and microsomal fractions to varying concentration of NEM, an alkylating agent, which reacts with sulfhydryl groups is shown in Table 4. 50% inhibition in mitochondrial and microsomal form of enzyme was observed at 20 mM and 12.5 mM concentration of NEM, respectively. Mitochondrial form of the enzyme appears to be more resistant to NEM action.

Table 5 shows the effect of DTT (0.5 to 40.0 mM) on the cholinephosphotransferase activities in mitochondria and microsome. DTT had inhibitory effect on both forms of enzyme. However, the mitochondrial form showed less inhibition (20% at 10 mM concentration) than microsomal one (25% inhibition at 5 mM concentration). But more than 70% of enzyme activity was retained even at a concentration as high as 40.0 mM in both mitochondrial and microsomal forms of enzyme.

Discussion

Cholinephosphotransferase is generally presumed to be located exclusively in the endoplasmic reticulum [4]. However, our laboratory had reported earlier the presence of the enzyme in the mitochondria of liver and lung of guinea pig and rat [15–19]. We further established that the enzyme is located in the outer mitochondrial membrane of guinea pig lung [17]. Recently, it has been reported by Vance [31] that in rat liver there is a subfraction of the endoplasmic reticulum that is specifically associated with mitochondria and this membrane fraction referred as 'fraction X' has cholinephosphotransferase activity. This author has suggested that this membrane fraction may be involved in the transfer of lipid to and from the mitochondria.

Table 3. The effect of trypsin on the activity of cholinephosphotransferase in 55 day old fetal guinea pig lung mitochondria and microsomes

Fraction	Specific activity	
	− Trypsin	+ Trypsin
Mitochondria	1.41 ± 0.34	0.47 ± 0.06
Microsomes	0.31 ± 0.07	0.09 ± 0.02

The reactions were carried as indicated in 'methods' with the exception that 10 μl of trypsin was added to the assay mixture. The concentration of trypsin was 40 μM. Each value represents mean ± S.E.M. of four experiments. The specific activity is expressed as nmoles/min/mg of protein.

Shore and Tata [32] have earlier isolated a mitochondria-endoplasmic reticulum complex (rapidly sedimenting endoplasmic reticulum) from rat liver that sediments at low speed. It contains mitochondrial and microsomal marker enzymes and phospholipid-synthesizing enzymes [33]. We have previously reported that in guinea pig lung the specific activity of cholinephosphotransferase is highest in a membrane fraction intermediate in density between that of mitochondria and microsomes [15–17] and the intermediary fraction is of outer mitochondrial origin [17]. It appears that our previously reported intermediate fraction (20,000 × g pellet) in guinea pig lung [17] is similar to the recently described fraction X in rat liver [31]. However, even though there is a possibility that in rat liver there may be a fraction intermediate in density between mitochondria and endoplasmic reticulum which may be associated with mitochondria, we have confirmed the authenticity of the cholinephosphotransferase in outer mitochondrial membrane of guinea pig lung [15–19].

The activity of mitochondrial and microsomal cholinephosphotransferase differed to some extent in substrate specificity as well as in sensitivity to NEM and DTT. Although the diacylglycerol specificity of this enzyme in microsome has been studied by several investigators [6–8, 13, 24–26], their results have been complex and varied.

The reverse reaction of cholinephosphotransferase has been used to measure specificity towards endogenous 1,2-diacylglycerols in rat liver and lung microsomes [34–37]. In rat liver microsome, it was reported that cholinephosphotransferase utilized endogenous 1,2-diacylglycerols differing in their degree of unsaturation without marked selectivity. Furthermore, studies on substrate specificities of

Table 4. The effect of N-Ethylmaleimide on the activity of cholinephosphotransferase in the 55 day old fetal guinea pig lung mitochondria and microsomes

Concentration (mM)	Specific activity	
	Mitochondria	Microsomes
0	1.45 ± 0.16	0.44 ± 0.03
1.0	1.24 ± 0.17	0.35 ± 0.03
5.0	1.21 ± 0.18	0.34 ± 0.05
10.0	1.17 ± 0.10	0.25 ± 0.04
15.0	1.00 ± 0.06	0.21 ± 0.05
20.0	0.74 ± 0.08	0.20 ± 0.02
25.0	0.67 ± 0.10	0.20 ± 0.04
30.0	0.67 ± 0.04	0.18 ± 0.01
40.0	0.47 ± 0.04	0.17 ± 0.05

These reactions were carried as described in 'methods' with the exception that different concentrations of NEM were added to the incubation mixture. Each value represents the mean ± S.E.M. of five experiments. Specific activity is expressed as nmol/min/mg of protein.

Table 5. The effect of dithiothreitol on the activity of cholinephosphotransferase in the 55 day old fetal guinea pig lung mitochondria and microsomes

Concentration (mM)	Specific activity	
	Mitochondria	Microsomes
0	1.69 ± 0.11	0.52 ± 0.10
0.5	1.65 ± 0.06	0.42 ± 0.01
1.0	1.61 ± 0.09	0.45 ± 0.04
2.0	1.51 ± 0.03	0.41 ± 0.07
4.0	1.44 ± 0.08	0.40 ± 0.04
8.0	1.62 ± 0.05	0.43 ± 0.05
10.0	1.31 ± 0.16	0.38 ± 0.03
16.0	1.35 ± 0.13	0.38 ± 0.06
20.0	1.36 ± 0.03	0.40 ± 0.08
40.0	1.34 ± 0.04	0.39 ± 0.01

These reactions were carried as described in 'methods' with the exception that the incubation mixture contained different concentrations of DTT. Each value represented as the mean ± S.E.M. of four experiments. The specific activity is expressed as nmoles/min/mg of protein.

lung cholinephosphotransferase towards aqueously dispersed exogenous and endogenously generated diacylglycerols showed that pulmonary cholinephosphotransferase had a profound preference for unsaturated diacylglycerols and phosphatidylcholine [13, 24, 25].

We observed a significant difference in the utilization of unsaturated diacylglycerols and saturated diacylglycerols in both the mitochondrial and microsomal forms of enzyme. Among the mixed diacylglycerols, 1-palmitoyl-2-oleoyl-sn-glycerol appears to be best utilized. The microsomal form of enzyme showed no significant difference in specific activity between 1-palmitoyl 2-oleoyl-sn-glycerol and other unsaturated diacylglycerols.

It should be noted that some of the discrepancy in the results of several investigators may be attributed to various factors, such as varying animal models, differences in diacylglycerol concentrations, differences in the solubility of lipids as well as presence of oxidized fatty acids and 1,3 isomer of diacylglycerols as contaminants in 1,2-diacylglycerols.

The K_m for 1,2-dilinolenoyl-sn-glycerol in both forms varies significantly than that for either 1,2-dioleoyl-sn-glycerol or 1-palmitoyl-2-oleoyl-sn-glycerol. The concentration of CDP-choline necessary for maximal activity for mitochondrial and microsomal forms of enzyme was 60 μM and 80 μM, respectively. The concentration for rabbit lung microsome was observed to be 67 μM [24]. Similarly, the V_{max} values were significantly varied between different diacylglycerols for both mitochondrial and microsomal forms of enzyme which indicates that the affinity of this enzyme for different substrates varies between two forms.

Cholinephosphotransferase was inhibited by the addition of trypsin in both mitochondria and microsome (Table 3). Although this enzyme is thought to be tightly bound to microsomal membrane [14], recently Vance and Vance [21] suggested that this enzyme in endoplasmic reticulum and Golgi are exposed to the cytoplasmic surface. They found almost 98% inhibition by trypsin. Our result also showed the presence of this enzyme on outside of membrane in both endoplasmic reticulum and mitochondria.

In the present study differential response to NEM provided a criterion by which fetal lung mitochondrial and microsomal cholinephosphotransferase could be distinguished. Exposer of both mitochondrial and microsomal forms of the enzyme to NEM showed 60–70% inhibition at 40 mM concentration (Table 4). However, a greater inhibition of microsomal enzyme (53%) than the mitochondrial enzyme (31%) was observed at 15 mM concentration. This may suggest that the content of sulfhydryl group in active site varies between the two forms. However, since the enzymes are not purified, it is not known how much the SH reagents truly react with the enzyme SH groups.

Data on the effect of DTT on the activity of cholinephosphotransferase in mitochondria and microsome (Table 5) indicate that the enzyme of both forms is sensitive to disulfhydryl reducing reagents. However, the mitochondrial form showed less inhibition than microsomal form. It has been reported by Woodard *et al.* [38] that DTT causes 95% inhibition of phosphatidylcholine synthesis at only 10 mM concentration, but it does not have any effect on the cholinephosphotransferase in the synthesis of platelet activating factor. The differential susceptibility of mitochondrial and microsomal cholinephosphotransferase to DTT further indicate that there is a difference in the disulfide group in the active site of both forms of enzymes.

The results of this study indicate that fetal lung mitochondria contain a cholinephosphotransferase which differs from the microsomal enzyme to some extent in terms of its sensitivity to diacylglycerols, NEM and DTT. However, we cannot exclude at this time that such small differences may be caused by differences in the environment of the enzyme in two subcellular fractions, solubility of substrate diacylglycerols or interactions between diacylglycerols and membranes, rather than the enzymes per se. Furthermore, these experiments were carried out with microsome and mitochondria from whole lung. Further clarification of this problem must await the use of type II cells of the lung rather than whole lung which may be derived from any or all of the 40 cell types present in lung tissue [39, 40]. Alveolar type II cells are responsible for surfactant synthesis and are known to have an increased ca-

pacity to synthesize phosphatidylcholine [11]. Furthermore, type II cells can utilize endogenous disaturated diacylglycerols for phosphatidylcholine biosynthesis [11]. More specific conclusions can be made as to the contribution of cholinephosphotransferase to the integriety of the lung if specific cells are isolated and assayed for mitochondrial and microsomal activity. Furthermore, the purification of the enzyme is necessary to prove conclusively whether these are isozymes or different enzymes. However, the results of this study are extremely useful for understanding some fundamental properties and the distribution of cholinephosphotransferase in lung subcellular fractions.

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