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PHOSPHOLIPID TRANSFER ACTIVITIES IN MORRIS HEPATOMAS AND THE SPECIFIC CONTRIBUTION OF THE PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN

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Summary

Phospholipid transfer activities for phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were measured in three hepatomas of increasing growth rate and degree of dedifferentiation, the hepatomas 7787, 9633 and 7777, and compared to the activities found in normal and host liver. A 2—3-fold increase was found in the phosphatidylcholine and phosphatidylinositol transfer activities in the fast-growing 7777 hepatoma, while these activities were moderately or not increased in the 7787 and 9633 hepatomas. Phosphatidylethanolamine transfer was found to be extremely low in all three hepatomas. The possible significance of these findings with respect to the altered phospholipid content and composition of the hepatoma membranes is discussed.

The contribution of the phosphatidylcholine specific exchange protein to the total phosphatidylcholine transfer activity was determined in normal and host liver and in the hepatomas 7777 and 9633 with the aid of a phosphatidylcholine exchange protein specific antiserum. To this end a new procedure for the purification of the phosphatidylcholine exchange protein from rat liver was developed which leads to a final purification factor of 5300 and a high overall yield of 17%. In addition, this protein was chemically and immunologically characterized and its properties were compared to those of the bovine phosphatidylcholine exchange protein purified in our laboratory previously.

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Introduction

Isotopic labeling studies and studies with purified subcellular fractions have established that the endoplasmic reticulum is the main site of phospholipid biosynthesis in eukaryotic cells [1—5]. Proteins present in the cytosol fraction of tissue homogenates and capable of catalyzing transfer of phospholipids in vitro have been implicated in the intracellular transport of phospholipids from their site of synthesis to membranes not capable of de novo lipid synthesis [6]. As yet, no direct proof for such a role is available, although there is indirect evidence suggesting a link between phospholipid exchange activities and phospholipid biosynthesis in developing mouse lung [7] and in rat brain [8].

In our laboratory we are investigating the possible relationship between phospholipid metabolism and phospholipid exchange proteins in three Morris hepatomas of various growth rates. Many abnormalities in composition and total content of phospholipids of intracellular and plasma membranes of hepatomas are known [9—15]. Recently, a report appeared describing the presence of a tumor specific exchange protein capable of transporting all phospholipids. This protein was held responsible for the observed 'chemical dedifferentiation' of the hepatoma membranes [16]. In order to quantitate the levels of different exchange proteins in the various Morris hepatomas we are currently raising antisera against all known phospholipid exchange proteins from rat liver. Here we report on the purification of the phosphatidylcholine specific exchange protein, some of its characteristics in comparison to the phosphatidylcholine exchange protein from bovine liver and its contribution to the total phosphatidylcholine transfer activity in normal liver and hepatomas, as determined by the use of antisera. In addition, data are presented on the phosphatidylinositol and phosphatidylethanolamine transfer activities in normal and host liver and in the Morris hepatomas.

Materials and Methods

Materials. DEAE and CM-cellulose were obtained from Whatman Ltd (Maidstone, U.K.), Sephadex G-50 fine, agarose A and Ficoll from Pharmacia (Uppsala, Sweden) and hydroxyapatite (Bio-gel HTP) from Bio-Rad Laboratories (Richmond, CA). Freund's complete adjuvans was from Miles Laboratories (Stoke Poges, U.K.).

Assays of phospholipid exchange. Phosphatidylcholine and phosphatidylinositol transfer were measured with the microsome-liposome assays as described in previous publications [17,18]. Phosphatidylethanolamine transfer was measured by using the procedure of Bloj and Zilversmit [19] with some modifications. [^{14}C]Phosphatidylethanolamine was purified from the livers of rats killed 1 h after intraperitoneal injection of 50 μCi of 2-amino[2-^{14}C]-ethanol by DEAE-cellulose chromatography as described by Rouser et al. [20]. Egg phosphatidylcholine was obtained from Makor (Jerusalem). Lipid phosphorus was determined by using the method of Rouser et al. [21]. Beef heart mitochondria were isolated by using the procedure of Blair [22]. The mitochondria were not preheated and the non-exchangeable marker was omitted. Instead, after the incubation the mitochondria and liposomes were separated

by centrifugation (10 min at 11 000 × *g*) through a cushion of 14% (w/w) sucrose. The mitochondrial pellets were dissolved in a small volume of 10% (w/v) sodium deoxycholate and the radioactivity measured by liquid scintillation counting in toluene counting fluid [17] to which some Bio-Solve (Beckmann, Futterton, CA) was added to obtain a homogeneous medium.

Purification of phosphatidylcholine exchange protein from bovine liver. This was achieved by following the procedure of Kamp and Wirtz [17] and Wirtz et al. [23].

Purification of phosphatidylcholine exchange protein from rat liver. Livers from Wistar rats were stored at −20°C until use. A buffer of 0.25 M sucrose, 0.01 M Tris-HCl (pH 7.4) and 0.001 M EDTA) was added. After thawing, the livers were rinsed several times with the same buffer and a 30% (w/v) homogenate was prepared. The homogenate was centrifuged in a WKF (model G 50 K) centrifuge at 7500 rev./min for 20 min. The supernatant was adjusted to pH 5.1 with 3 N HCl and allowed to stand for 30 min with stirring after which the precipitated proteins and microsomes were removed by centrifugation as above. The supernatant was adjusted to pH 7.2 with 1 M Tris and subsequently, solid ammonium sulfate was added to 60% saturation. After standing overnight with stirring, precipitated proteins were sedimented by centrifugation as above and the pellet dissolved in a small volume of 10 mM Tris-HCl, pH 7.2. After dialysis against 10 mM Tris-HCl (pH 7.2) and removal of denatured proteins by centrifugation this protein was applied to a DEAE-cellulose column (50 mg protein/ml DEAE cellulose) equilibrated and eluted with the same buffer. The void volume fraction containing the exchange protein was adjusted to 90% saturation with solid ammonium sulfate, allowed to stand overnight and the precipitated protein was sedimented by centrifugation as above. The pellet was dissolved in a small volume of 20 mM citrate/40 mM phosphate buffer, pH 5.0, and dialyzed extensively against this buffer. After removal of precipitated protein this fraction was applied to a CM-cellulose column (15 mg protein/ml CM-cellulose) equilibrated with 20 mM citrate/40 mM phosphate buffer, pH 5.0. The protein was eluted with a linear gradient of 20 mM citrate/40 mM phosphate, pH 5.0, to 80 mM citrate/160 mM phosphate, pH 5.0 (2 × 5 column volumes). The active fractions eluted at a buffer strength of about 35 mM citrate/70 mM phosphate. After adjustment of the pH to 6.8 with 3 N NaOH the pooled fractions were concentrated against dry Ficoll and applied to a Sephadex G-50 column (6 × 80 cm) equilibrated with 10 mM potassium phosphate, pH 6.8. The phosphatidylcholine exchange protein eluted at V_e/V_o = 1.8. Final purification was obtained by applying the active fractions to a hydroxylapatite column (1—5 mg protein/ml hydroxyapatite). The protein was eluted with a linear gradient of 10 mM potassium phosphate to 300 mM potassium phosphate, pH 6.8 (2 × 5 column volumes). Pure phosphatidylcholine exchange protein eluted at about 180 mM potassium phosphate. Protein concentrations were determined by using the method of Lowry et al. [24] with bovine serum albumin as standard.

Determination of purity and molecular weight of the phosphatidylcholine exchange protein. To determine the purity of the phosphatidylcholine exchange protein, polyacrylamide gel electrophoresis in acid buffer as described by Gabriel [25] and sodium dodecyl sulfate gel electrophoresis as described by

O'Farrell [26] were employed. Sodium dodecyl sulfate gels, 12.5% in acrylamide, were used for the molecular weight determination. α-Lactalbumin (M_r 14 400), trypsin inhibitor (M_r 20 100), carbonic anhydrase (M_r 30 000), ovalbumin (M_r 43 000) and albumin (M_r 67 000) obtained from Pharmacia and cytochrome *c* (M_r 12 500), papain (M_r 23 400) and bovine serum albumin (M_r 68 000) obtained from Boehringer (Mannheim, F.R.G.) were used as standard proteins.

Amino acid analysis. Aliquots of 2 nmol of lyophilized phosphatidylcholine exchange protein were hydrolyzed in 6 N HCl in vacuo at 110°C for 24, 48 and 72 h. The amino acid composition was determined on a Technicon TSM amino acid analyzer. Cysteic acid was determined after peroxidation with performic acid [27].

Immunization procedure and purification of IgG fractions. New Zealand white rabbits (weight 2.5 kg) were immunized with a 2 ml emulsion of 1 vol. of isotonic saline containing 600 μg rat liver phosphatidylcholine exchange protein and 1 vol. of Freund's complete adjuvant. Portions of 200 μl were injected intradermally in the back and the shoulders. After 7 weeks a booster injection of 300 μg phosphatidylcholine exchange protein in isotonic saline was given subcutaneously. If necessary, the booster injection was repeated once after an additional 2 weeks. Usually, the rabbits were exsanguinated 2—2½ months after the primary immunization. Pre-immune and immune sera were obtained by allowing the blood to clot for 1 h at 37°C and the clot to retract at 4°C. After centrifugation to remove the residual red blood cells the sera were dialyzed against 15 mM potassium phosphate buffer, pH 8.0, and applied to a DEAE-cellulose column (1 ml serum/5 ml DEAE cellulose) equilibrated with the same buffer. The IgG fraction, which elutes in the void volume, was stored in small aliquots at −20°C.

Double immunodiffusion on 1% agarose gels was performed according to the of procedure Ouchterlony and Nilsson [28].

Hepatomas. Morris hepatomas 7787, 9633 and 7777 were obtained from Dr. K.Y. Hostetler (University of California, San Diego, U.S.A.). These are chemically induced transplantable hepatomas of increasing growth rate and degree of dedifferentiation as defined in Refs. 29 and 30. The tumors were maintained in Buffalo strain rats and were transplanted by injection of 0.5—1.0 ml of a tumor mince in Hank's balanced salt solution into the hind limbs of the animals. The tumors were harvested after 3 weeks (hepatoma 7777), 3 months (hepatoma 9633) and 4—6 months (hepatoma 7787), respectively.

Preparation of 105 000 × g and pH 5.1 supernatants from rat liver hepatoma. Male rats (250 g) were fasted overnight and the liver and/or tumor was removed. A 10% (w/v) homogenate in 0.25 M sucrose, 0.01 M Tris-HCl (pH 7.4), 0.001 M EDTA buffer was prepared and was subsequently centrifuged for 5 min at 1000 × *g*, 10 min at 20 000 × *g* and 90 min at 105 000 × *g*. The 105 000 × *g* supernatant was adjusted to pH 5.1 with 1 N HCl and allowed to stand for 30 min on ice. Precipitated protein was removed by centrifugation and the pH adjusted to 7.4 with 1 M Tris.

Inhibition studies. Purified rat and beef liver phosphatidylcholine exchange protein and 105 000 × *g* supernatants of liver and Morris hepatomas were incubated for 30 min at 37°C with increasing concentrations of pre-immune and

anti-phosphatidylcholine exchange protein IgG. 1% bovine serum albumin (w/v) was added to the incubations with the pure proteins and 0.2% with the supernatants to prevent denaturation during the incubation. After the incubation the tubes were put on ice for 2—3 h and subsequently assayed for phosphatidylcholine exchange activity for 30 min at 37°C.

Results

Purification and characterization of the phosphatidylcholine exchange protein from rat liver

Table I gives a summary of the purification procedure. A final purification factor of 5300 was obtained and the overall recovery was 17% with respect to the pH 5.1 supernatant. Starting with 48.8 g of pH 5.1 supernatant protein, which is equivalent to 1.5 kg rat liver, one obtains about 1.5 mg of pure phosphatidylcholine exchange protein. This protein is specific for phosphatidylcholine; no phosphatidylinositol or phosphatidylethanolamine transfer activity could be measured with the assay systems described above. On DEAE-cellulose and polyacrylamide electrophoresis in acid buffer it behaves like a basic protein. This is in accordance with the isoelectric point of 8.4 previously published [31,32]. The final protein fraction was homogeneous as judged by both polyacrylamide electrophoresis and sodium dodecyl sulfate gel electrophoresis. The molecular weight was estimated to be 28 000 and was virtually the same as that of the bovine phosphatidylcholine exchange protein (see Fig. 1). This value is higher than the molecular weight of 16 000 found by Lumb et al. [31].

The amino acid compositions of the phosphatidylcholine exchange protein are given in Table II, column A. For comparison the amino acid compositions of the rat liver phosphatidylcholine exchange protein purified by Lumb et al. [31], recalculated on the basis of a molecular weight of 28 000 and of the bovine protein published by Moonen et al. [33] are included (columns B and C, respectively). Since very few differences were found between the amino acid compositions determined after 24, 48 and 72 h of hydrolysis, our data represent an average value. The amino acid composition of our protein is similar to that published by Lumb et al. [31] with the exception of a higher serine and a lower histidine content. In spite of the fact that the bovine phosphatidylcholine exchange protein is acidic, the amino acid composition of the rat and

TABLE I

PURIFICATION OF PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN FROM RAT LIVER

	Protein (mg)	Specific activity (% transport/mg protein)	Recovery (%)	Purification (-fold)
pH 5.1 supernatant	48 800	4.9	100	1
60% ammonium sulfate	21 480	7.7	70	1.6
DEAE-cellulose	5 880	20.0	50	4.1
90% ammonium sulfate, pH → 5.0	4 920	26.3	54	5.4
CM-cellulose	1 240	74.8	27	15.3
Sephadex G-50	16.2	2 580	17.5	526
Hydroxyapatite	1.54	26 000	17.0	5300

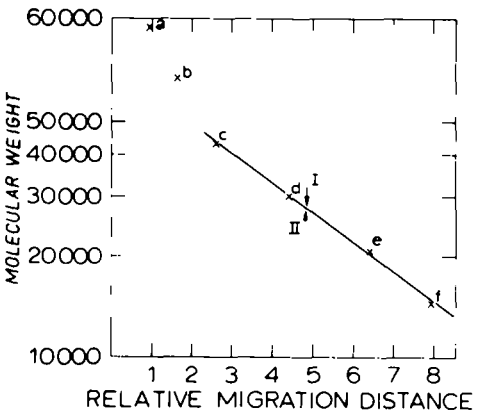


Fig. 1. Semilogarithmic plot of molecular weight vs. relative migration distance of standard proteins and rat (I) and bovine (II) phosphatidylcholine exchange proteins. The following standard proteins were used: phosphorylase *b* M_r 94 000 (a), bovine serum albumin M_r 67 000 (b), ovalbumin M_r 43 000 (c), carbonic anhydrase M_r 30 000 (d), trypsin inhibitor M_r 20 000 (e) and α-lactalbumin M_r 14 400 (f).

bovine proteins are rather similar (columns A and C). However, the rat liver protein has a much higher serine, glycine and histidine content. Smaller differences are observed in the tyrosine, leucine and isoleucine contents.

Immunization of rabbits with the rat liver phosphatidylcholine exchange protein resulted in a very active highly specific antiserum. Fig. 2 shows the

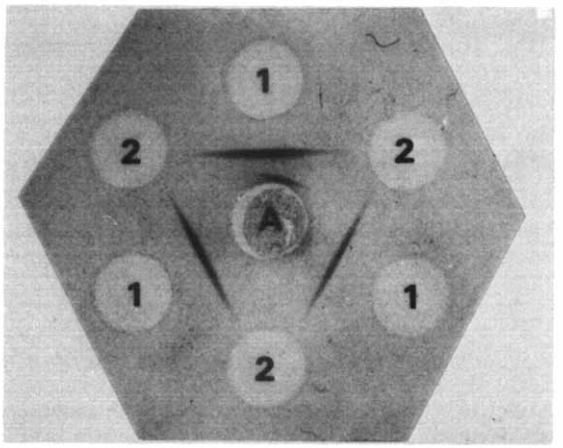
TABLE II

AMINO ACID COMPOSITION OF PURIFIED RAT LIVER PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN (A). COMPARISON WITH PREVIOUSLY PUBLISHED DATA ON RAT (B) AND BOVINE (C) PHOSPHATIDYLCHOLINE EXCHANGE PROTEINS

n.d., not determined.

Amino acid	A	B *	C **
Lysine	20	23	20
Histidine	6	10	3
Arginine	9	10	10
Tryptophan	n.d.	n.d.	4
Aspartic acid and asparagine	21	19	21
Threonine	11	9	7
Serine	30	23	15
Glutamic acid and glutamine	32	28	35
Proline	13	12	13
Glycine	28	26	17
Alanine	14	17	17
Half cystine	3	3—4	4
Valine	17	14	21
Methionine	4	4	5
Isoleucine	11	9	6
Leucine	15	16	21
Tyrosine	9	9	15
Phenylalanine	8	9	10
Total	251	242	244

* Ref. 31.
** Ref. 33.



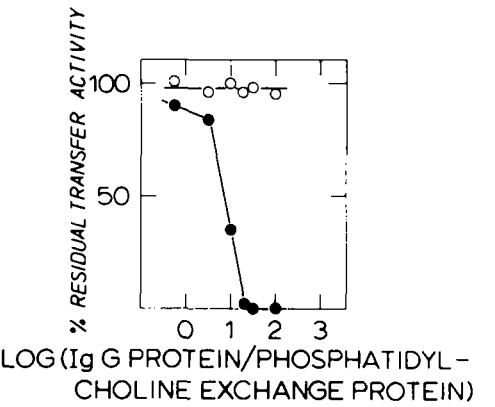


Fig. 2. Double-immunodiffusion experiment according to the method of Ouchterlony and Nilsson [28]. The centre well contained 150 μg of anti-rat liver phosphatidylcholine exchange protein IgG. Wells 1 contained 7.5 μg rat liver phosphatidylcholine exchange protein and wells 2 7.5 μg of bovine liver phosphatidylcholine exchange protein.

Fig. 3. Effect of anti-rat liver phosphatidylcholine exchange protein IgG on the activities of rat (●——●) and bovine (○——○) phosphatidylcholine exchange proteins. 3 μg of exchange protein were incubated with increasing amounts of IgG and assayed for activity as described in Materials and Methods. Results are expressed as % residual activity vs. log (IgG/exchange protein).

result of a double-immunodiffusion experiment with the purified IgG fraction from anti-rat liver phosphatidylcholine exchange protein antiserum and the rat and beef liver phosphatidylcholine exchange proteins. With the rat liver proteins a single precipitatin line is seen. The absence of a precipitin line with the beef liver protein shows that the IgG fraction is not cross-reactive. With pH 5.1 supernatants from rat liver also one single precipitin line was observed which showed a reaction of identity with pure rat liver protein. No precipitin line was observed with IgG from preimmune serum (results not shown).

Fig. 3 shows the results of an inhibition experiment where fixed amounts of phosphatidylcholine exchange protein from rat and beef liver were incubated with increasing amounts of anti-rat liver phosphatidylcholine exchange protein antiserum prior to assaying for exchange activity. Complete inhibition of the rat liver protein transfer activity was seen at an IgG protein/exchange protein ratio of 20 : 50 (w/w). The activity of the beef liver protein was not inhibited, confirming the results of the double-immunodiffusion experiment. These data show that we have obtained a very active antiserum which is highly specific for the rat liver phosphatidylcholine exchange protein.

Phospholipid transfer activities in hepatomas

Phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine transfer activities were measured with 105 000 × *g* supernatants from liver of control rats, host liver and Morris hepatomas 7777, 9633 and 7787. The activities as presented in Table III are expressed relative to normal liver. No significant difference was observed between host and normal liver in any of the three phospholipid transfer activities measured. Phosphatidylcholine and phospha-

TABLE III

PHOSPHOLIPID TRANSFER ACTIVITIES IN 105 000 × *g* SUPERNATANTS OF MORRIS HEPATOMAS 7777, 9633, 7787 AND HOST LIVERS AS COMPARED TO NORMAL LIVER

Phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine transfer activities were measured as described in Materials and Methods. Specific transfer activities are expressed in arbitrary units relative to normal liver.

	Phosphatidylcholine transfer activity	Phosphatidylinositol transfer activity	Phosphatidylethanolamine transfer activity
Normal liver	100	100	100
Host liver	120	110	90
Hepatoma 7777	255	190	5
Host liver	110	110	80
Hepatoma 7787	170	130	15
Host liver	105	100	90
Hepatoma 9633	95	120	10

tidylinositol transfer activities were increased 2.5- and 2-fold, respectively, in the supernatant of the fast-growing 7777 hepatoma. The activities were not increased or only to a moderate degree increased in the supernatants of the slow-growing 9633 and 7787 hepatomas. Most interestingly, the phosphatidylethanolamine transfer activity, as measured with the mitochondria-liposome assay was virtually absent in all three tumors.

The anti-rat liver phosphatidylcholine IgG has been used to determine the relative contribution of the phosphatidylcholine exchange protein to the total phosphatidylcholine transfer activity in normal liver, 7777 and 9633 hepatomas (Fig. 4). From the results it can be seen that the phosphatidylcholine exchange protein contributes 50% to the activity in rat liver (Fig. 4A). No difference was observed between host liver and normal liver. In the 7777 hepatoma, phosphatidylcholine exchange protein accounted for 60% of the total phosphatidylcholine transfer activity (Fig. 4B). Thus, the transfer activity of the phosphatidylcholine exchange protein in the 7777 supernatant is increased to approximately the same degree as the total phosphatidylcholine transfer

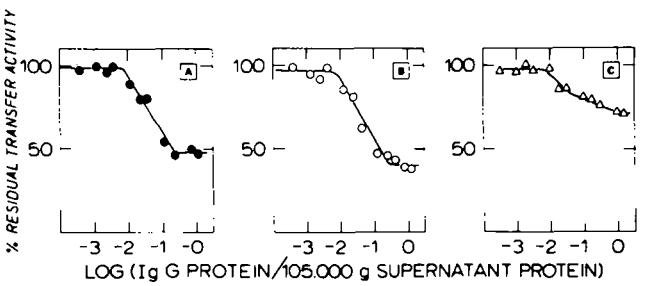


Fig. 4. Effect of anti-rat liver phosphatidylcholine exchange protein IgG on the phosphatidylcholine transfer activity in 105 000 × *g* supernatants of normal rat liver (A) and hepatomas 7777 (B) and 9633 (C). 2.5 mg of supernatant protein were incubated with increasing amounts of IgG and assayed for activity as described in Materials and Methods. Results are expressed as % residual activity vs. log (IgG/supernatant protein).

activity. A different pattern is seen in the 9633 hepatoma (Fig. 4C). Here the phosphatidylcholine exchange protein activity comprises only 30% of the total phosphatidylcholine transfer activity and there is a specific decrease both in relative and absolute amounts of phosphatidylcholine exchange protein as compared to normal and host liver (see also Table III).

Discussion

In this publication data are presented on the phospholipid exchange protein activities in Morris hepatomas of various growth rates with an emphasis on the activity of the phosphatidylcholine specific exchange protein. To determine the contribution of this protein to the total phosphatidylcholine transfer activity, an antiserum against this protein has been raised. In a previous study we reported on the use of an antiserum against the phosphatidylcholine exchange protein from bovine liver [34]. This antiserum was not cross-reactive with the phosphatidylcholine exchange protein in the 105 000 $\times g$ supernatant from rat liver. Therefore, we developed a procedure to purify the phosphatidylcholine specific exchange protein from rat liver in order to obtain a specific antiserum. This procedure yields a homogeneous protein which was purified 5300-fold, and leads to a much higher recovery of phosphatidylcholine exchange protein than the method previously published (17% compared to 2%, Ref. 31). With this protein we obtained a very active antiserum which was highly specific; no cross-reactivity with the bovine liver phosphatidylcholine exchange protein was found despite similarities in amino acid composition, molecular weight and phospholipid exchange specificity.

Although all eukaryotic cells contain phospholipid exchange proteins, little is known about their physiological role. Our hypothesis is that in vivo these proteins act as carriers of phospholipids between the endoplasmic reticulum and membranes not capable of de novo phospholipid synthesis, for example, mitochondria [6]. Membrane alterations in phospholipid content and composition could possibly be related to changes in levels and relative distribution of phospholipid exchange proteins. The well described changes in the phospholipid content and composition of mitochondria from hepatomas prompted us to investigate this relationship. Table IV gives the data obtained by Hostetler et al. [15] on the content of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine in mitochondria of normal liver and of 7777, 7787 and 9633 Morris hepatomas. A doubling of the phosphatidylcholine content in

TABLE IV

PHOSPHOLIPID CONTENT OF MITOCHONDRIA OF NORMAL LIVER AND HEPATOMAS 7777, 7787 AND 9633

Results are expressed as nmol lipid phosphorus/mg protein and are the means ± S.D. Results are taken from Ref. 15.

Phospholipid	Normal liver	7777	7787	9633
Phosphatidylcholine	66.9 ± 7.0	126.0 ± 14.0	99.5 ± 1.5	70.9 ± 2.7
Phosphatidylinositol	15.5 ± 1.4	30.4 ± 7.6	15.3 ± 1.1	11.1 ± 1.4
Phosphatidylethanolamine	64.9 ± 6.5	107.0 ± 16.0	106.0 ± 18.0	88.8 ± 14.5

the mitochondria of the fast-growing 7777 hepatoma, a moderate increase, and no increase in the well-differentiated hepatomas 7787 and 9633, respectively, were observed. As for the phosphatidylinositol content, only the mitochondria of the 7777 hepatomas demonstrated a 2-fold increase. It is of interest that the levels of phosphatidylcholine and phosphatidylinositol exchange protein activity in these tumors parallel these changes in phospholipid content (cf. Table III).

The 2.5-fold increase in the phosphatidylcholine transfer activity in Morris 7777 hepatoma led us to investigate whether this increase was due to the phosphatidylcholine exchange protein. Inhibition studies with the anti-rat liver phosphatidylcholine exchange protein IgG demonstrated that the relative contribution of this protein was very similar in liver and in this hepatoma. This implies that in absolute terms the phosphatidylcholine exchange protein has increased 2.5-fold in Morris 7777 hepatoma. Although in the 9633 hepatoma the total phosphatidylcholine transfer activity is similar to normal liver, only 30% could be attributed to the phosphatidylcholine exchange protein. It remains to be established which exchange proteins are responsible for the remaining phosphatidylcholine transfer activity found in these hepatomas.

Phosphatidylethanolamine transfer activities were very low in all three hepatomas compared to normal and host liver (Table III).

It is of interest that this activity is low, irrespective of the growth rate and degree of differentiation of these tumors. Phosphatidylethanolamine transfer activity is most probably due to the non-specific (phospho)lipid exchange protein described by Bloj and Zilversmit [19]. Low levels of this protein could be transformation-linked as is suggested for another small molecular weight binding protein, the fatty acid binding protein [34]. As is seen from Table IV, phosphatidylethanolamine levels are increased in the mitochondria of all three hepatomas. So, contrary to those of phosphatidylcholine and phosphatidylinositol, the levels of phosphatidylethanolamine do not parallel the levels of the phosphatidylethanolamine transfer activity. If the non-specific exchange protein is involved in the transport of phosphatidylethanolamine, this protein is apparently not required in hepatomas. One explanation could be that tumor mitochondria have an independent way of synthesizing phosphatidylethanolamine which is not present in normal liver. This was actually shown to be the case for the mitochondria of the 7777 hepatoma which can synthesize phosphatidylserine by base-exchange, a pathway normally only active in the endoplasmic reticulum [36]. This newly formed phosphatidylserine can be decarboxylated to phosphatidylethanolamine by the mitochondrial phosphatidylserine decarboxylase. Low levels of non-specific exchange protein could also have some bearing on the intracellular cholesterol transport, since this protein is also capable of transferring cholesterol between membranes in vitro [19]. This is of particular interest in view of the abnormal control of cholesterol synthesis which is a common feature of most hepatomas [34].

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