PHOSPHOLIPID TRANSFER PROTEIN FROM BOVINE RETINA WITH HIGH ACTIVITY TOWARDS RETINAL ROD DISC MEMBRANES

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1. Introduction

Phospholipid transfer proteins have been isolated from a number of tissues (reviewed in [1]). These proteins, which range from 12 500–25 000 mol. wt, catalyze the transfer of phospholipid from a donor such as endoplasmic reticulum or artificial unilamellar bodies (liposomes) to an acceptor such as mitochondria. The most widely studied transfer protein is found in the cytosol of bovine liver and has high activity towards phosphatidyl choline [2–7], although transfer proteins in other tissues with preferences for phosphatidyl choline [8,9], other phospholipids [2,9–11], and cholesterol [2,12] have been described. We have identified a phosphatdiyl choline transfer protein in the cytosol of bovine retina that is active toward bovine retinal rod outer segment (ROS) disc membranes, and have compared it to the soluble transfer protein from bovine liver.

2. Materials and methods

[^{14}C]Triolein was purchased from Amersham Searle and purified by thin-layer chromatography immediately before use. Radioactive phospholipids were obtained by injecting rats with $^{32}PO_4$ (New England Nuclear) and extracting and fractionating liver phospholipids [2]. Purity was determined by two dimensional thin-layer chromatography [13] and phospholipid mass quantitated by phosphorus assay [14] after chromatography. Liposomes were prepared by adding together 2.5 mgm [^{32}P]phosphatidyl choline, 25 μg butylated hydroxytoluene and 8 μg [^{14}C]triolein in 0.4 ml buffer containing 0.25 M sucrose, 1 mM Na_2EDTA and 50 mM Tris–HCl, pH 7.4 (SET buffer). The mixture was allowed to swell 1 h at room temperature and then was sonicated at 20°C for 30 min [2], or until the suspension cleared. SET buffer and 20 mg bovine serum albumin (fraction IV powder, Miles Labs) were added to the sonicated liposomes to final vol. 4 ml.

Soluble fractions from bovine retina and liver containing phosphatidyl choline transfer protein were prepared by acid and $(NH_4)_2SO_4$ precipitation according to the standard procedure in [15]. Retina ROS were prepared by the procedures in [16] or [18]. All membrane fractions were stored frozen in SET buffer.

ROS and mitochondrial membrane preparations containing equimolar amounts of phosphatidyl choline were placed in individual incubation tubes, to which was added an aliquot of [^{32}P]phosphatidyl choline liposomes and the pH 3.0 soluble fraction containing transfer protein. SET buffer was added to final vol. 0.5 ml and tubes were incubated for specified times in air at 37°C with gentle agitation in a Dubnoff Metabolic Incubator. Donor liposomes were separated from acceptor membranes by centrifugation at 10 000 × g for 10 min, and ^{14}C and ^{32}P dpm determined in the liposomes that remained in the supernatant. Recovery of triolein in the supernatant

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was always greater than 85%, the activity not recovered reflecting non-specific binding of the liposomes to ROS or the incubation tube. Knowing the ^{32}P and ^{14}C dpm in the liposomes prior to incubation, and the % recovery of ^{14}C, the predicted dpm of ^{32}P in the liposomes was calculated assuming no phospholipid transfer. The difference between the predicted and the observed values was the total dpm of phosphatidyl choline transferred between liposomes and rod outer segments or mitochondrial membranes. The net transfer mediated by the transfer proteins was determined by subtracting the total dpm transferred in the presence and absence of transfer proteins. Because of the rapid decay of ^{32}P, the data are expressed as % transfer of ^{32}P phospholipid.

3. Results

The bovine retina contains a soluble protein that transfers phosphatidyl choline from unilamellar liposomes to bovine heart mitochondria and bovine ROS membranes. Transfer is dependent on time and protein concentration. As has been observed for the liver protein [1], transfer of phosphatidyl choline by the retina protein is abolished by *N*-ethyl malemide.

Figure 1 shows the time course of phosphatidyl choline transfer from liposomes to mitochondria (A) and ROS (B) by the soluble retina fraction. This fraction is more active towards ROS than towards mitochondria, the acceptor usually used in assays for transfer proteins. In all of our studies, the ratio of soluble protein mass to liposomal phospatidyl choline to acceptor membrane phosphatidyl choline was kept constant, so the data reflect a true preference of the transfer protein for ROS membranes as acceptors of phosphatidyl choline.

Figure 2 is the time course of transfer of phosphatidyl choline from liposomes to bovine heart mitochondria (A) and ROS (B) by the bovine liver soluble fraction. Like the retina preparation, the liver fraction is more active towards ROS than mitochondria as an acceptor for phospholipid transfer. The retina transfer protein has about half the activity of the liver protein, based on the % of phosphatidyl choline transferred/mg protein.

The transfer of phosphatidyl choline from liposomes to ROS and mitochondria also is demonstrated by the data in table 1. Not only are ROS a preferred acceptor compared to mitochondria, there also appears to be a difference in the specificity of the retina and liver transfer proteins. In two separate experiments employing different preparations of liver and retina transfer proteins as well as acceptor mem-

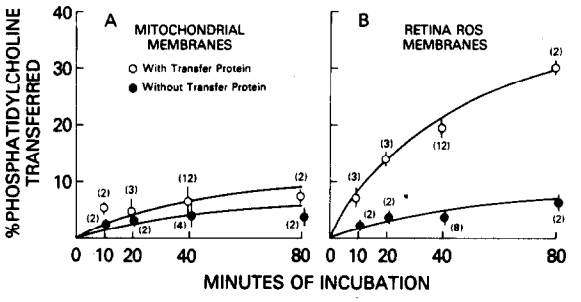

Fig.1. Time course of percent transfer of phosphatidyl choline (PC) between unilamellar liposomes and (A) bovine heart mitochondria and (B) bovine ROS by a soluble protein fraction from bovine retina. Each incubation tube contained 32.5 μg liposomal PC, 108 μg acceptor membrane PC and 1.7 mg pH 3.0 soluble protein made to 0.5 ml with SET buffer. The values in parentheses are the number of separate determinations at each time. The bar represents one standard deviation on either side of the mean.

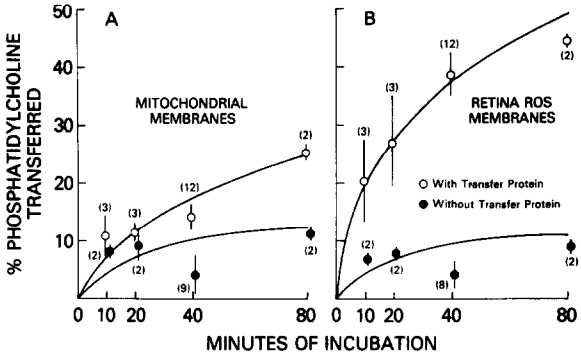

Fig.2. Time course of percent transfer of phosphatidyl choline (PC) between unilamellar liposomes and (A) bovine heart mitochondria and (B) bovine ROS by a soluble protein fraction from bovine liver. Each incubation tube contained 32.5 μg liposomal PC, 108 μg acceptor membrane PC and 1.7 mg pH 3.0 soluble protein made to 0.5 ml with SET buffer. The values in parentheses are the number of separate determinations at each time. The bar represents one standard deviation on either side of the mean.

Table 1
Transfer of phosphatidyl choline from liposomes to natural membranes during 40 min. of incubation[a]

	Source of transfer protein	Percent transfer of phosphatidyl choline		Percent net transfer of phosphatidyl choline		Ratio ROS/mitochondria
		ROS membrane	Mitochondria membrane	ROS membrane	Mitochondria membrane	
A.	Retina	18.9 ± 2.1[b]	9.8 ± 0.7	14.4[c]	2.7	
		20.0 ± 1.2	7.7 ± 0.5	12.9	2.9	
		21.6 ± 1.6	–	15.6	–	
				$\bar{x}$ = 14.3	$\bar{x}$ = 2.8	5.1
	Liver	33.4 ± 1.3	14.6 ± 0.8	28.9	7.8	
		36.6 ± 2.5	13.1 ± 0.6	29.5	8.3	
		39.9 ± 1.7	–	33.9	–	
				$\bar{x}$ = 30.8	$\bar{x}$ = 7.9	3.9
	None (control)	4.5 ± 0.5	7.1 ± 0.7			
		7.1 ± 0.7	4.8 ± 1.1			
		6.0 ± 2.3	–			
B.	Retina	17.8 ± 1.2	5.5 ± 1.2	15.4	3.8	
		22.0 ± 2.4	4.6 ± 0.8	18.6	2.2	
				$\bar{x}$ = 17.0	$\bar{x}$ = 3.0	5.7
	Liver	38.8 ± 0.9	12.4 ± 2.1	36.4	10.7	
		42.6 ± 1.8	13.1 ± 0.9	39.2	10.7	
				$\bar{x}$ = 37.8	$\bar{x}$ = 10.7	3.5
	None (control)	2.4 ± 1.1	1.7 ± 0.7			
		3.4 ± 0.8	2.4 ± 0.6			

[a] A and B are different preparations of acceptor membranes and pH 3.0 soluble fractions from liver and retina. Each tube contained 32.5 μg liposomal PC, 108 μg acceptor membrane PC, and 1.7 mg pH 3.0 soluble protein made to 0.5 ml with SET buffer

[b] Each value is the mean ± SD of quadruplicate determinations. Preparation A was assayed on three separate occasions and preparation B on two

[c] These values represent net transfer, obtained by subtracting nonspecific transfer which occurs in the absence of added protein (control) from total phospholipid transferred

branes, the retina protein was 5.1 and 5.7-times more active toward ROS than toward mitochondria while the liver protein was 3.9 and 3.5-times as active.

4. Discussion

The ROS of vertebrate visual cells contain thousands of membranous discs composed of nearly equal parts lipid and protein. In mammalian rods, the protein components of the ROS are renewed every 9–10 days. Proteins synthesized in the inner segment are incorporated into new discs at the base of the outer segments, while older discs near the apical tips are shed and phagocytized by the pigment epithelium. Once incorporated into a disc, rhodopsin molecules remain there until the disc is shed (reviewed in [20]).

Little is known about the renewal of ROS lipid. It was found [21] that labeled lipid precursors were incorporated randomly into ROS, leading them to suggest that whereas ROS protein is renewed by membrane replacement, the lipid is renewed by both membrane and molecular replacement. Lipid renewal by either mechanism implies transport of newly synthesized lipid from its synthetic site, the smooth endoplasmic reticulum of the inner segment, to the site of incorporation into the membrane at the base of the outer segments. It has been suggested that the

role of phospholipid transfer proteins may be to mediate this type of lipid transfer [22]. The cellular origin of the retinal phosphatidyl choline transfer protein we have described is not known. Studies are now in progress to determine if the protein is in the rod visual cell, where it could be involved in phospholipid transfer between the inner segment and ROS discs.

We have found bovine rod outer segments to be better acceptors of phosphatidyl choline than bovine heart mitochondria for both liver and retina transfer proteins. One possible explanation is the high levels of long-chain polyunsaturated fatty acids (predominantly 22:6ω3) in rod outer segments [23]. It has been shown [12] that transfer of liposomal cholesterol to red blood cells is dependent upon the degree of unsaturation of phospholipid fatty acids in the donor phosphatidyl choline/cholesterol vesicles. However, other possibilities that must be considered are differences in membranes architecture, the degree of interaction of intrinsic membrane proteins with membrane phospholipids and the symmetry of the distribution of phosphatidyl choline in the two membranes.

A further suggestion from the data in table 1 is that while both proteins are active in transferring phosphatidyl choline to ROS membranes, the retina appears to contain a protein that is more specific than the liver for ROS. This may indicate an intrinsic difference in the proteins from these two tissues.

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