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A STUDY ON PHOSPHOLIPID EXCHANGE PROTEINS PRESENT IN THE SOLUBLE FRACTIONS OF BEEF LIVER AND BRAIN

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SUMMARY

1. A soluble fraction of beef liver denoted as pH 5.1 supernatant stimulated the exchange of various phospholipids between rat liver mitochondria and microsomes in the order: phosphatidylcholine > phosphatidylinositol > phosphatidylethanolamine. With a pH 5.1 supernatant isolated from beef brain, the order of stimulation was: phosphatidylinositol > phosphatidylcholine. The latter supernatant did not stimulate the exchange of phosphatidylethanolamine.

2. A rabbit antiserum was prepared against a purified phosphatidylcholine exchange protein isolated from a beef liver pH 5.1 supernatant. The γ-globulin fraction of this serum completely inhibited the activity of this phosphatidylcholine exchange protein. In contrast, the γ-globulin fraction only partially inhibited the phosphatidylcholine exchange activity present in beef liver and brain pH 5.1 supernatants. The phosphatidylinositol and phosphatidylethanolamine exchange activities present in these supernatant fractions were not markedly affected by treatment with the γ-globulin fraction.

3. It is concluded that in addition to the phosphatidylcholine exchange protein against which the γ-globulin fraction of the antiserum is active, other phospholipid exchange activities, possibly proteins, are present in the soluble fractions of beef liver and brain.

Exchange of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine between subcellular membranes such as those of mitochondria and microsomes is well documented[1–5]. A cytosol fraction of the cell obtained after pH 5.1 treatment of a high speed cell supernatant stimulated the exchange of these phospholipids[6]. It was found that pH 5.1 supernatant fractions from various tissues of the rat stimulated the exchange of each phospholipid class to a different extent[7]. In the present study similar results were obtained with the pH 5.1 supernatant fractions from beef brain and liver (see Table I). We recently reported the purification of a phospholipid exchange protein from beef liver which specifically stimulated the exchange of phosphatidylcholine[8]. This specificity was apparent in the phospholipid exchange between mitochondrial and microsomal membranes from rat liver as well as between one of these membranes and liposomes.

These observations suggested that specific proteins may be involved in the exchange of each phospholipid class. Immunological evidence is presented in this report that, in addition to the phosphatidylcholine exchange protein discussed above, other phospholipid exchange activities are present in the pH 5.1 supernatant fractions from beef liver and brain. The pH 5.1 supernatant fractions and phosphatidylcholine exchange protein of beef liver were isolated as previously described[8,9]. Membrane preparations, extraction and radiochemical analyses were also carried out as described before[9].

Phospholipid exchange activity was assayed by two methods (Assay A and B). In Assay A, activity of the phosphatidylcholine exchange protein only was determined by the *in vitro* transfer of [^{14}C]phosphatidylcholine from rat liver microsomes labelled with [^{14}C]phosphatidylcholine to liposomes (see legend to Fig. 1). In Assay B, activities with respect to the exchange of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine were determined simultaneously by the *in vitro* transfer of these phospholipids from ^{32}P-labelled rat liver microsomes to unlabelled mitochondria of the same tissue (see legend to Table I).

Antiserum against the phosphatidylcholine exchange protein was obtained from rabbits as previously described[9]. A γ-globulin fraction which was shown to have antibody activity towards the phosphatidylcholine exchange protein was prepared by saturation of the serum to 18% (w/v) with solid sodium sulphate. The precipitate was collected by centrifugation, dissolved in water and dialysed against distilled water until the salt was removed. After dialysis the protein solution was lyophilised and the lyophilised material stored at -10 °C. A control γ-globulin fraction was obtained from rabbits that had not been immunized with the exchange protein, in a similar way. Fresh solutions of these γ-globulin fractions were prepared in 0.25 M sucrose–0.001 M EDTA–0.01 M Tris (pH 7.4) before reaction with the antigen. The antigen–antibody reaction was performed by incubation of phosphatidylcholine exchange protein or pH 5.1 supernatant fractions with the antibody* in a total volume of 2 ml sucrose–EDTA–Tris for 30 min at 37 °C and a subsequent storage for two days at 4 °C. A precipitate was formed which was removed by centrifugation at 15000 rev./min for 20 min in the SS 34 rotor of the Sorvall centrifuge. Phospholipid exchange activities were determined in the supernatant fractions. Controls consisted of phosphatidylcholine exchange protein or pH 5.1 supernatant fractions which were incubated without antibody or with the control γ-globulin fraction (1 mg per 20 mg supernatant protein or 2 μg exchange protein). It was found that both types of control gave similar results.

The effect of increasing amounts of antibody on the stimulation of phosphatidylcholine exchange by pure phosphatidylcholine exchange protein is shown in Fig. 1. The phosphatidylcholine exchange activity was determined by Assay A and is given as percent microsomal [^{14}C]phosphatidylcholine transferred to the liposomes per μg phosphatidylcholine exchange protein. The results show that complete inactivation of the exchange protein occurred at a concentration of 8 μg exchange protein per mg antibody. Below this equivalence point no exchange activity was found: conversely above 8 μg exchange protein, exchange activity was observed

* The term antibody which is used in the text, refers to the γ-globulin fraction of the antiserum.

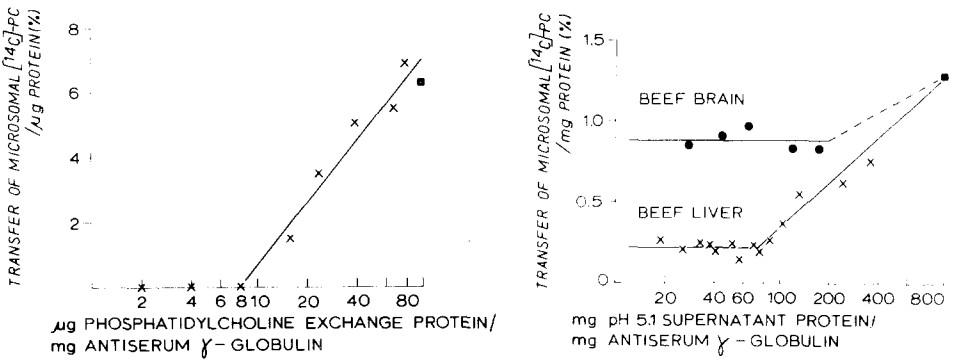

Fig. 1. Inhibition of activity of phosphatidylcholine exchange protein by antibody against phosphatidylcholine exchange protein. Antibody (0.5 mg by weight) was incubated with different amounts of phosphatidylcholine exchange protein as described in text. After removal of the antigen–antibody precipitate, supernatant was assayed for phosphatidylcholine exchange activity as previously described[9]. Microsomes labelled with [^{14}C]phosphatidylcholine (2.5 mg protein, *a* ^{14}C cpm) were incubated with liposomes containing trace of [^{3}H]cholesteryloleate (1 μmole phospholipid P, *b* ^{3}H cpm) and aliquots of supernatant containing the phosphatidylcholine exchange protein, in 2.5 ml sucrose–EDTA–Tris (pH 7.4) for 30 min at 25 °C. At the end of incubation the ^{14}C/^{3}H ratio (X) of the liposomal lipid extract was calculated per μg phosphatidylcholine exchange protein. Percentage of [^{14}C]phosphatidylcholine ([^{14}C]-PC) which is transferred from microsomes to liposomes is $(b\ X/\ a) \times 100\%$. The percent transfer is corrected for a blank incubation. ×—×, activity of phosphatidylcholine exchange protein after treatment with antibody. ■, control without antibody.

Fig. 2. Inhibition of phosphatidylcholine exchange activity in pH 5.1 supernatant fractions from beef liver and brain by antibody against phosphatidylcholine exchange protein. Antibody (0.5 mg by weight) was incubated with different amounts of pH 5.1 supernatant protein from beef liver and brain as described in text. Phosphatidylcholine exchange activity was determined (see legend Fig. 1). ×—×, phosphatidylcholine exchange activity of beef liver pH 5.1 supernatant after teatment with antibody; ●—●, phosphatidylcholine exchange activity of beef brain pH 5.1 supernatant after treatment with antibody; ■, controls without antibody of beef liver and brain pH 5.1 supernatant fractions. [^{14}C]-PC, [^{14}C]phosphatidylcholine.

rising to the control value of about 6.3% transfer per μg protein. These results indicate that the phosphatidylcholine exchange protein from beef liver is antigenic and that the antibody–antigen interaction completely inactivates this exchange protein.

In a similar experiment, the effect of the antibody on the phosphatidylcholine exchange activity present in the pH 5.1 supernatant fractions from beef liver and brain was determined using Assay A (Fig. 2). The equivalence point of the antigen–antibody reaction with beef liver pH 5.1 supernatant was at about 70 mg supernatant protein per mg antibody. It is seen, however, that below this equivalence point the phosphatidylcholine exchange activity is not inhibited completely. With Assay A a residual activity of about 15% was observed compared to the control value. Above the equivalence point the exchange activity increases. In contrast, the phosphatidylcholine exchange activity of beef brain supernatant was inhibited to a much lesser extent by the antibody. The residual activity was about 70% of the control value. An equivalence point for this latter supernatant was not determined.

The residual phosphatidylcholine exchange activity may be due to a less

effective interaction of the antibody with the antigen in those crude pH 5.1 supernatant fractions. Alternatively the results of the experiments illustrated in Figs 1 and 2 could suggest that the antibody inhibits one particular phosphatidylcholine exchange protein that is present to a greater extent in the liver than in the brain. Whether the remaining phosphatidylcholine exchange activity of these liver and brain supernatant fractions reflects a second exchange protein remains to be established. It is noted that Ehnholm and Zilversmit[10] have isolated two proteins from beef heart which differed in molecular weight and isoelectric point, but which both stimulated the exchange of phosphatidylcholine.

Various pH 5.1 supernatant–antibody mixtures as represented in Fig. 2 were simultaneously assayed for phosphatidylcholine, phosphatidylinositol and phosphaditylethanolamine exchange activity using Assay B (Table I). The control of beef brain supernatant stimulated the exchange of phosphatidylinositol to a greater extent than that of phosphatidylcholine (3.9% transfer *versus* 1.3% per mg supernatant protein, respectively), whereas the exchange of phosphatidylethanolamine was not stimulated at all. Treatment of the beef brain supernatant with excess antibody inhibited the phosphatidylcholine exchange activity by 20–30% but had no effect on the phosphatidylinositol exchange activity. The control of beef liver supernatant stimulated the exchange of phosphatidylcholine slightly better than that of phosphatidylinositol (2.0% transfer *versus* 1.7%, respectively, per mg supernatant protein)

TABLE I

INHIBITION OF EXCHANGE ACTIVITIES OF BEEF LIVER AND BRAIN pH 5.1 SUPERNATANT FRACTIONS IN RESPECT OF INDIVIDUAL PHOSPHOLIPIDS BY ANTIBODY AGAINST PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN

pH 5.1 supernatant–antibody mixtures as represented in Fig. 2 were assayed for phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine exchange activity as previously described[8]. ^{32}P-labelled microsomes (2.5 mg protein) were incubated with unlabelled mitochondria (12.5 mg protein) and aliquots of supernatant–antibody mitures in 2.5 ml sucrose–EDTA–Tris (pH 7.4) for 30 min at 25 °C. At the end of incubation mitochondria were isolated, phospholipids extracted and ^{32}P radioactivity of each phospholipid class determined. The same procedure was followed with non-incubated ^{32}P-labelled microsomes. From the ^{32}P radioactivity, percent transfer of each [^{32}P]phospholipid class from microsomes to mitochondria was calculated per mg supernatant protein. Percent transfer is corrected for blank incubation.

Supernatant fraction	*Supernatant/ antibody ratio* *	*Transfer of [^{32}P]phospholipid per mg pH 5.1 supernatant protein (%)* **		
		Phosphatidyl-choline	*Phosphatidyl-inositol*	*Phosphatidyl-ethanolamine*
Beef brain	control	1.26	3.90	—
	176	0.90 (71%)	4.00 (103%)	—
	47	1.03 (82%)	4.21 (108%)	—
Beef liver	control	2.03	1.74	0.23
	57	0.81 (40%)	1 93 (111%)	0.22 (96%)
	23	0.73 (36%)	1.34 (77%)	0.23 (100%)

* Numbers refer to numbers on the abscissa of Fig. 2.

** Numbers between parentheses give percent activity relative to control value.

whereas the exchange of phosphatidylethanolamine was stimulated to a much lesser extent (0.2% per mg supernatant protein). After treatment of the beef liver supernatant with excess antibody, a maximal inhibition of approximately 60% was observed for the phosphatidylcholine exchange activity whereas the phosphatidylinositol and phosphatidylethanolamine exchange activities were virtually unaffected. These results demonstrate the specificity of the antibody with respect to the inhibition of the phosphatidylcholine exchange activity. Additional evidence for the specific inhibition by the antibody is presented in Fig. 3. Various amounts of beef liver pH 5.1 supernatant protein after treatment with excess antibody show a 45% reduction in phosphatidylcholine exchange activity. As before, the phosphatidylinositol and phosphatidylethanolamine exchange appeared to be independent of antibody treatment.

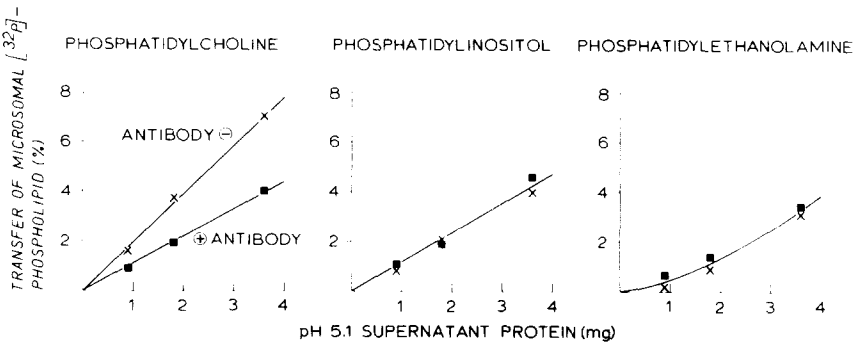

Fig. 3. Effect of antibody against phosphatidylcholine exchange protein on exchange activities of beef liver pH 5.1 supernatant fraction in respect of invidual phospholipids. A pH 5.1 supernatant–antibody mixture as represented in Fig. 2 (19 mg supernatant protein per mg antibody) was assayed for phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine exchange activity (see legend to Table I). Activity was determined for various amounts of protein of this mixture. Exchange activities of pH 5.1 supernatant treated with a control γ-globulin fraction were also determined. Incubations were at 37 °C for 30 min. ×—×, activity after treatment with control γ-globulin fraction; ■—■, activity after treatment with antibody.

From these results it may be concluded that, in addition to the phosphatidylcholine exchange protein against which the antibody is active, other activities in respect of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine exchange are present in beef liver and brain supernatants. Whether these activities reflect a series of exchange proteins which are specifically involved in the exchange of each phospholipid class is currently under investigation. Preliminary results suggest that a protein fraction can be isolated from beef brain which stimulates, with a high degree of specificity, the *in vitro* exchange of phosphatidylinositol (Harvey, Helmkamp and Wirtz, unpublished results).

It appears from Figs 2 and 3 and Table I that the level of residual phosphatidylcholine exchange activity of beef liver supernatant after optimal inhibition with antibody depends on the assay used. The residual exchange activity amounted to 15% with Assay A and approximately 50% with Assay B. At the moment we do not know the cause of this variation. It may reflect the difference in receptor membranes

used in the assays, namely liposomal in Assay A and mitochondrial in Assay B. In both assays, *in vivo* labelled rat liver microsomal membranes prepared by identical procedures were used as the donor membrane.

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