Biochimica et Biophysica Acta, 691 (1982) 37–43
Elsevier Biomedical Press

BBA 71334

KINETICS OF PROTEIN-MEDIATED TRANSFER OF RAT PANCREATIC MICROSOMAL PHOSPHATIDYLINOSITOL TO LIPOSOMES

KRIT PATUMRAJ, ANDREW GOULD, SACHITHANA SUBRAMANIAM and FRANK SLABY

Department of Anatomy, George Washington University Medical Center, 2300 I Street, N.W., Washington, DC 20037 (U.S.A.)

(Received April 28th, 1982)

Key words: Phosphatidylinositol; Phospholipid exchange protein; Microsome; Liposome; (Rat pancreas)

Pancreatic microsomes were isolated from fasted and pilocarpine-injected rats and the microsomal phosphatidylinositol radiolabelled with *myo*-[2-^{3}H]inositol by isotopic exchange. A standard reaction mixture was established in which partially purified rat liver phosphatidylinositol exchange proteins sustain a maximal rate of phosphatidylinositol transfer from rat pancreatic microsomes to liposomes. Determination of the transfer kinetics shows (1) that pancreatic microsomal phosphatidylinositol is partitioned approximately equally between a non-exchangeable and a single exchangeable pool and (2) that cholinergic stimulation does not significantly change the relative sizes of the two pools nor the exchange half-life of the latter pool.

Introduction

Cytosolic phospholipid exchange proteins have been purified from liver and brain tissue which preferentially exchange phosphatidylinositol (PI) between membrane systems under in vitro conditions [1,2]. Brophy et al. [3] demonstrated in a study of the kinetics of protein-mediated PI transfer from rat liver microsomes to liposomes that bovine PI exchange proteins can distinguish three distinct pools of PI in these microsomes: One pool is not subject to protein-mediated exchange; the other two pools are both exchangeable but are exchanged with significantly different kinetics. They have suggested that the rapidly and slowly exchangeable pools represent free and protein-bound PI, respectively, residing in the outer (or cytoplasmic) phospholipid layer of the microsomal membranes and that the non-exchangeable pool represents PI residing in the inner (or luminal) layer.

The purpose of the study presented here was to compare microsomes isolated from non-stimulated and cholinergically-stimulated rat pancreatic tissue with respect to (1) the kinetics of protein-mediated PI transfer to liposomes and (2) the relative proportions among the exchangeable and non-exchangeable PI pools. The study was conducted because in many cells there is a prompt and pronounced alteration of PI turnover in response to appropriate stimuli [4]. In particular, increased PI turnover occurs during stimulated secretion in a wide variety of exocrine and endocrine protein-secreting cells [5–9]. In the exocrine pancreatic acinar cell, cholinergic stimulation of secretion elicits within 2 min a several-fold increase in the rate of PI synthesis and by 40–60 min a 33–40% net decrease in the PI content [10–12]. The rough endoplasmic reticulum is the site of most of the increased PI synthesis and degradation [13]. Since microsomes isolated from pancreatic tissue represent primarily the vesiculated remains of the exocrine cell rough endoplasmic reticulum, we sought

Abbreviations: PI, phosphatidylinositol; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; DEAE-cellulose, diethyl amino ethyl-cellulose.

0005-2736/82/0000-0000/$02.75 © 1982 Elsevier Biomedical Press

to determine if cholinergic stimulation of rat pancreatic exocrine cells is associated not only with changes in the turnover of the PI in the rough endoplasmic reticulum, but also with changes in the exchangeability of pancreatic microsomal PI.

Materials and Methods

Preparation of rat pancreatic microsomes. Pancreatic tissue was excised from Sprague-Dawley rats (weighing 150–250 g) sacrificed by decapitation. The tissue was excised from animals subjected to one of three experimental conditions: (1) The rats were fasted overnight (16–24 h) prior to sacrifice. (2) The rats were fasted overnight and intraperitoneally injected with 10 mg/kg body weight of the cholinergic drug, pilocarpine hydrochloride, 1 h prior to sacrifice. (3) The rats were fasted overnight and provided with standard laboratory chow for 2–3 h prior to sacrifice. In this last experimental condition, the animals were individually housed to confirm that each animal consumed 2–3 pellets of food during the 2–3 h feeding period.

Pancreatic tissue was immersed in ice-cold buffer A (0.25 M sucrose, 1 mM EDTA and 10 mM Tris-HCl, pH 7.4) upon excision, fat and mesentery trimmed away, and the tissue minced with scissors. The medium was drained from the tissue mince and the tissue homogenized with a Teflon/glass Potter-Elvehjem homogenizer in fresh ice-cold buffer A (3 ml buffer A per gram tissue). The 25% (w/v) homogenate was centrifuged at 14000 rpm ($15300 \times g_{av}$) for 15 min at 2°C in a Beckman JA-20 rotor to sediment nuclei, zymogen granules, mitochondria and heavy microsomes. The supernatant was centrifuged at 45000 rpm ($189000 \times g_{av}$) for 45 min at 2°C in a Beckman SW50.1 rotor to sediment the microsomes. The microsomal pellets were homogenized in 20 mM Tris-HCl, pH 7.4, and the microsomal PI radiolabelled with *myo*-[2-^{3}H]inositol by isotopic exchange according to the procedure of Helmkamp et al. [1]. In each separate experiment, all the microsomes (5–7 mg microsomal protein) isolated from a single pancreas were incubated at 37°C for 2 h in 5.0 ml 10 mM $MnCl_2$ and 20 mM Tris-HCl, pH 7.4, containing 12.5 μC_i *myo*-[2-^{3}H]inositol (1.0 mCi/0.078 μmol *myo*-inositol) to radiolabel the microsomal PI; this radiolabelling procedure yielded microsome preparations having approx. $3 \cdot 10^6$ dpm per mg microsomal protein. In four separate experiments we determined that 70–75% of the microsomal protein, phospholipid and PI initially present in the microsome suspension (prepared from either fasted or pilocarpine-injected rats) were recovered after radiolabelling the microsomal PI and washing the microsomes free of unincorporated *myo*-[2-^{3}H]inositol. The radiolabelling procedure thus does not significantly alter the relative proportions of these microsomal components. We also measured 16, 17 and 18 μg PI per mg microsomal protein in three separate microsome suspensions prepared from fasted rats; there were 14, 14 and 15 μg PI per mg microsomal protein in three separate microsome suspensions prepared from pilocarpine-injected rats. The substantial decrease in cellular PI content which occurs during cholinergic stimulation of pancreatic exocrine cells is therefore represented as only an average 15% decrease in the PI content of pancreatic tissue microsomes.

Preparation of rat liver microsomes. Livers were excised from rats fasted overnight and sacrificed by decapitation. Microsomes were isolated and the microsomal PI radiolabelled with *myo*-[2-^{3}H]inositol as described above.

Preparation of partially purified rat liver PI exchange proteins. Rat liver PI exchange proteins were partially purified according to a modification of the procedure of Lumb et al. [2]. Liver post-microsomal supernatants were prepared from 25% (w/v) homogenates of 2–3 rat livers as described above and dialyzed against 5 mM sodium phosphate, pH 7.4. The dialyzed supernatant was centrifuged at 14000 rpm for 15 min at 2°C in a Beckman JA-20 rotor and the supernatant applied to a 1.6×15 cm column of DEAE-cellulose equilibrated with 5 mM sodium phosphate, pH 7.4. The column was then washed with 20 ml 5 mM sodium phosphate, pH 7.4, and 40 ml 25 mM NaCl and 5 mM sodium phosphate, pH 7.4, and the eluants discarded; the first eluant contains phosphatidylcholine exchange protein [2]. 30 ml 50 mM NaCl and 5 mM sodium phosphate, pH 7.4, were applied to the column and the collected eluant saturated to 90% with ammonium sulfate and stirred overnight. The precipitated protein was sedimented by centrifugation at 14000 rpm for 15

min at 2°C in a Beckman JA-20 rotor and then dissolved in 1–2 ml 5 mM sodium phosphate, pH 7.4. The resulting protein solution was dialyzed against 50 mM sodium phosphate, pH 7.4, and then assayed for PI exchange activity. Such partially purified PI exchange protein preparations contain two PI exchange proteins [2]. The preparations can be stored at 2°C for 48 h without loss of enzymatic activity.

Preparation of liposomes. Preparations of egg L-α-phosphatidylcholine and soybean L-α-phosphatidylinositol dissolved in chloroform were mixed (98 mol% phosphatidylcholine to 2 mol% PI), dried under vacuum at 25°C, redissolved in diethyl ether, and then dried again under vacuum at 25°C. The lipids were allowed to swell for 1–2 h at 2°C in buffer A and the suspension then sonified at 2°C. (Branson Sonifier Cell Disruptor 350; 50% duty cycle; output control at 2; four 0.5-min sonifications).

Assay of PI exchange activity. PI exchange activity was assayed by determining the in vitro transfer of phosphatidyl[2-^{3}H]inositol from rat liver microsomes to liposomes. As discussed above, the purpose of this study was to compare the exchangeability of pancreatic microsomal phosphatidyl[2-^{3}H]inositol in microsomes isolated from fasted, fed and pilocarpine-injected rats. Accordingly, the use of rat liver microsomes to measure PI exchange activity served to insure that the findings of the exchangeability of pancreatic microsomal PI would be independent of the procedure employed for the determination of PI exchange activity in our partially purified rat liver PI exchange protein preparations. Aliquots of partially purified PI exchange protein preparations were added to tubes containing *myo*-[2-^{3}H]inositol-labelled rat liver microsomes (150 μg microsomal protein), liposomes (0.5 μmol liposomal phospholipid) and buffer A to a final volume of 0.5 ml. After incubation at 37°C for 20 min, 2.0 ml ice-cold buffer A were added to each reaction mixture and the resulting suspension layered atop a step Metrizamide gradient in buffer A consisting of 1.0 ml 10% (w/v) Metrizamide. 1.0 ml 20% (w/v) Metrizamide and 0.5 ml 30% (w/v) Metrizamide. After centrifugation at 20000 rpm ($37000 \times g_{av}$) for 20 min at 2°C in a Beckman SW50.1 rotor, the top and bottom 2.5 ml were collected, each fraction mixed with 0.5 ml 5% (w/v) SDS and then Aquasol-2 and radioactivity determined with a Beckman liquid scintillation spectrometer.

The Metrizamide gradient centrifugation procedure separates liposomes and microsomes by taking advantage of the fact that the two types of membranous vesicles have significantly different average equilibrium densities. Metrizamide, a triiodinated benzamido-derivative of glucose, is a density gradient material which gives dense aqueous solutions of low viscosity and osmolality [14]; membranous organelles and vesicles rapidly attain their equilibrium density in Metrizamide gradients centrifuged at relatively low speeds [15,16]. The step Metrizamide gradient was designed to permit only the microsomes to equilibrate within the gradient, leaving the liposomes suspended in the layer atop the gradient. The following experiments were conducted to insure that the centrifugation procedure results in a consistently high recovery of liposomes and microsomes in the top and bottom 2.5 ml fractions, respectively, independent of the composition of the reaction mixture and the duration of the incubation period: (1) We found that if a suspension of liposomes labelled with cholesteryl[1-^{14}C]oleate is layered atop the step Metrizamide gradient and centrifuged as described above, 98–99% of the radioactivity is recovered in the top 2.5 ml fraction. This 98–99% liposomal recovery in the top fraction is also obtained after incubation (for periods as long as 3 h) of the cholesteryl[1-^{14}C]oleate-labelled liposomes with rat liver PI exchange proteins and microsomes. (2) We found that when suspensions of separate preparations of *myo*-[2-^{3}H]inositol-labelled rat liver microsomes are layered atop the gradient and centrifuged as described above, 75–85% of the radioactivity is recovered in the bottom 2.5 ml fraction (the percentage recovery is 70–80% for *myo*-[2-^{3}H]inositol-labelled rat pancreatic microsomes). These high percentages of microsomal recovery in the bottom fraction do not vary with respect to the about of microsomes (6–800 μg microsomal protein) layered atop the gradient. Incubation of *myo*-[2-^{3}H]inositol-labelled microsomes with either liposomes of partially purified rat liver PI exchange proteins results in no more than a 5% shift of the total radioactivity from the bottom to the

top fraction. (3) When duplicate aliquots of a *myo*-[2-^{3}H]inositol-labelled microsome supension are incubated with liposomes for identical periods of time, layered atop gradients, and then centrifuged as described above, the percentages of radioactivity recovered in the bottom 2.5 ml fraction of the two gradients vary by no more than one percentage point. Thus, although the centrifugation procedure is somewhat laborious, it does offer the advantage of providing highly reproducible microsomal recoveries in the bottom gradient fractions from reaction mixtures prepared with the same microsome suspension.

The percentage of microsomal phosphatidyl[2-^{3}H]inositol transferred to liposomes in an experimental reaction mixture was determined in all experiments as follows: Every experimental reaction mixture containing microsomes, liposomes and PI exchange proteins was co-incubated with a control reaction mixture containing only microsomes and liposomes. Following separation of the microsomes and liposomes by the Metrizamide gradient centrifugation procedure, the percentage of total *myo*-[2-^{3}H]inositol radioactivity recovered in the top fraction for each reaction mixture was determined. Let A and B represent the percentages of the total radioactivity recovered in the top fractions for the control and experimental reaction mixtures, respectively. A represents not only the precentage (15–30%) of the microsomes recovered in the top fraction for the reaction mixture in which there is no protein-mediated PI transfer to liposomes, but also the percentage of microsomes in the experimental reaction mixture for which protein-mediated transfer of radiolabelled PI to liposomes cannot be detected (as this radioactivity is also recovered in the top fraction). The term $(B - A)$ thus represents the percentage of radiolabelled microsomal PI in the experimental reaction mixture which was transferred to liposomes from the percentage of $(100 - A)$ of microsomes for which transfer of radiolabelled PI to liposomes can be detected. The percentage of microsomal phosphatidyl[2-^{3}H]inositol transferred to the liposomes in the experimental reaction mixture was therefore calculated by the formula $100 \cdot (B - A)/(100 - A)$.

Control experiments established that PI exchange activity as measured in the above assay procedure is directly proportional to enzyme content if 10% or less of the microsomal PI is transferred. This determination permitted us to define one unit of PI exchange activity as the transfer of 1% of the microsomal PI after 20 min incubation in the assay reaction mixture.

Analytical procedures. Phospholipids were extracted from membranous vesicles (either microsomes or liposomes) by mixing two volumes of the vesicle suspension with one volume ice-cold 2 mg bovine serum albumin per ml and then vortexing with one volume ice-cold 40% (w/v) trichloroacetic acid. The supensions were centrifuged at 10000 rpm ($7800 \times g_{av}$) for 5 min at 2°C in a Beckman JA-20 rotor. The ice-cold trichloroacetic acid pellets were washed once with ice-cold 5% (w/v) trichloroacetic acid and then suspended in 5.0 ml ice-cold chloroform/methanol (2:1, v/v). The suspensions were blended on a Vortex mixer with 1.05 ml 0.05 M KCl and cenrifuged at 14000 rpm for 10 min at 2°C in a Beckman JA-20 rotor. The upper phase was removed by aspiration and the lower phase washed twice with 2.5 ml theoretical upper phase (chloroform/methanol/0.05 M KCl, 3:48:47, v/v/v) before being dried under vacuum at 25°C.

The identity of phospholipids in the extracts containing incorporated *myo*-[2-^{3}H]inositol was established by thin layer chromatography on Analtech Silica gel G precoated plates developed by the two-dimensional system described by Yavin and Zutra [17]. Phospholipid content was measured by inorganic phosphate determination [18].

Protein concentration was determined according to the procedure of Lowry et al. [19] using bovine serum albmin as a standard.

Materials. All reagents used were of the highest purity available Egg yolk L-α-phosphatidylcholine, soybean L-α-phosphatidylinositol, bovine serum albumin and DEAE-cellulose were purchased from Sigma Chemical Co.; Metrizamide from Accurate Chemical and Scientific Corp., Hicksville, NY; and *myo*-[2-^{3}H]inositol and cholesteryl[1-^{14}C]oleate from New England Nuclear.

Results

In order to compare PI exchangeability in pancreatic microsomes isolated from non-stimulated

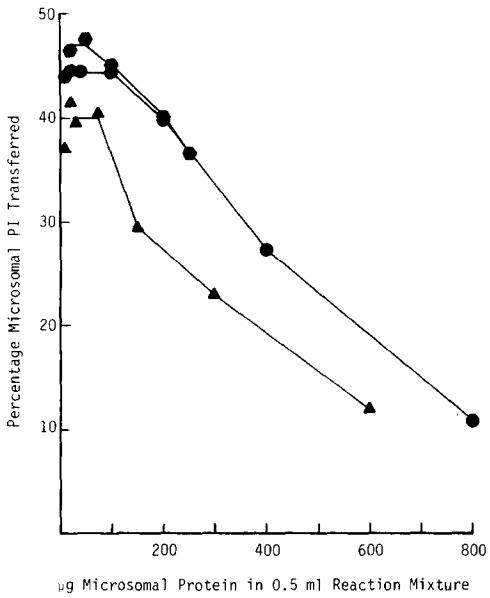

Fig. 1. Percentage of microsomal PI transferred to liposomes as a function of microsome concentration. Reaction mixtures containing 0.5 μmol liposomal phospholipid, 100 units of PI exchange activity and varying concentrations of microsomal protein were incubated for 15 min and the percentage of pancreatic or liver microsomal PI transferred determined as described in Materials and Methods. The pancreatic microsomes were isolated from starved (●——●) and pilocarpine-injected (■——■) rats and the liver microsomes (▲——▲) from starved rats.

and cholinergically-stimulated tissue, it was necessary to determine first a reaction mixture composition which sustains a maximal rate of microsomal PI transfer to liposomes. Fig. 1 shows the percentage of microsomal PI transferred in reaction mixtures containing 0.5 μmol liposomal phospholipid and 100 units of PI exchange activity as a function of microsome concentration. Under these conditions there is maximal PI transfer after 15 min incubation if 100 μg microsomal protein or less are in the reaction mixtures containing either pancreatic microsomes isolated from starved and pilocarpine-injected rats or liver microsomes isolated from starved rats. We therefore established that a reaction mixture containing 100 units of PI exchange activity, 0.5 μmol liposomal phospholipid and 20 μg microsomal protein in a total volume of 0.5 ml (hereafter referred to as the standard reaction mixture) will sustain a maximal rate of PI transfer from either pancreatic or liver microsomes to liposomes.

Table I shows the kinetics of PI transfer in the standard reaction mixture for (a) pancreatic microsomes isolated from animals subjected to the three experimental conditions described in Materials and Methods and (b) liver microsomes isolated from starved animals. Almost all of the exchangeable PI is transferred from each type of pancreatic

TABLE I

KINETICS OF PANCREATIC AND LIVER MICROSOMAL PI TRANSFER TO LIPOSOMES IN THE STANDARD REACTION MIXTURE

The results are expressed as the average ± S.D. The numbers in parentheses indicate the number of separate experiments conducted with each type of microsome preparation.

Tissue	Physiological condition of rats from which microsomes were isolated	Percentage of microsomal PI transferred after					
		5 min	10 min	15 min	30 min	60 min	180 min
Pancreas	Starved (9)	22.3 ±5.1	35.7 ±5.3	41.6 ±5.3	47.0 ±7.6		50.5 ±6.7
Pancreas	Fed (5)	28.4 ±5.1	41.5 ±5.7	48.1 ±5.7	51.4 ±3.1		53.5 ±2.1
Pancreas	Pilocarpine-injected (4)	29.1 ±4.3	42.1 ±5.8	48.1 ±6.2	55.9 ±8.3		59.0 ±8.7
Liver	Starved (4)	20.3 ±3.6	36.0 ±4.1	40.1 ±4.8	49.9 ±3.5	55.2 ±4.0	69.8 ±6.2

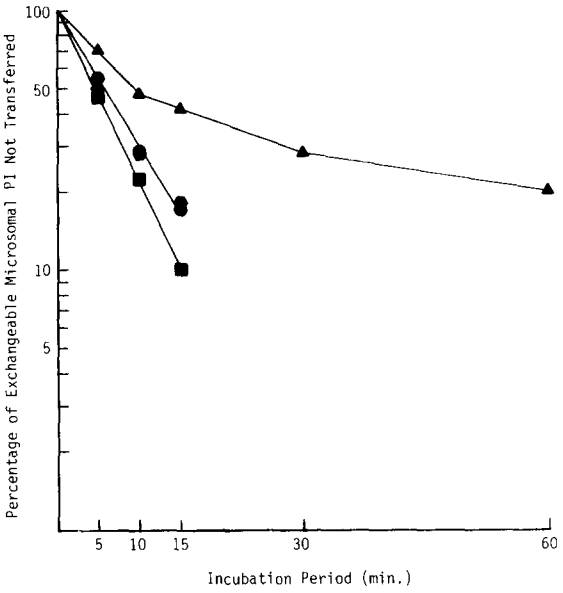

Fig. 2. Semi-logarithmic graphs of exchangeable microsomal PI during the first 15 min of incubation in a standard reaction mixture containing pancreatic microsomes isolated from starved rats (●———●), fed rats (■———■) and pilocarpine-injected rats (■———■) and during the first 60 min of incubation in a standard reaction mixture containing liver microsomes isolated from starved rats (▲———▲). The kinetic data shown in Table I were used in the determination of these graphs. The percentage of exchangeable microsomal PI not transferred at each time point was calculated by first subtracting the percentage of total microsomal PI transferred at the specified time point from the maximally exchangeable percentage measured after 3 h incubation. This figure was then divided by the maximally exchangeable percentage and multiplied by 100 to yield the percentage of exchangeable microsomal PI not transferred.

microsome preparation after 30 min incubation; the average maximum percentages of microsomal PI transferred after 3 h incubation are 51, 54 and 59% for pancreatic microsomes isolated from starved, fed and pilocarpine-injected rats, respectively. (Thin-layer chromatographic analysis showed that 85–90% of all the radioactivity extracted from both the pancreatic microsomes and liposomes after 3 h incubation in the standard reaction mixture co-chromatographs with authentic PI.) In contrast, only about 70% of the exchangeable PI is transferred from the liver microsomes after 30 min incubation. The kinetics of PI transfer from liver microsomes are characterized by a 15–30 min period of rapid transfer followed by an extended period of slow transfer; an average maximum 70% of the total microsomal PI is transferred after 3 h incubation.

Semi-logarithmic graphs of the exchange kinetics displated in Table I yield linear plots from the pancreatic microsome kinetic data but a non-linear plot from the liver microsome kinetic data (Fig. 2). The linearity of the pancreatic plots demonstrates that there is a single exchangeable PI pool in each of the three types of pancreatic microsomes. Statistical analysis indicates that the slopes of these linear plots are not significantly different, and, therefore, that all of the exchangeable PI in each type of pancreatic microsomes has an exchange half-life of 5–6 min. The non-linearity of the liver plot, on the other hand, indicates the presence of two or more exchangeable PI pools in liver microsomes. As Brophy et al. [3] have noted, the rapid phase and slow phase of protein-mediated PI transfer from liver microsomes suggest the presence of just two major exchangeable PI pools. On such an assumption, we can let the slope the liver plot between 30 and 60 min represent the exchange half-life of the slowly exchangeable pool and extrapolate this part of the plot to zero-time to estimate the percentage of exchangeable microsomal PI in this pool. Such analysis indicates that in our standard reaction mixture 40% of the liver microsomal PI is transferred with an exchange half-life of 5–6 min and 30% with an exchange half-life of 50–60 min.

Discussion

The findings reported here demonstrate that, under conditions in which rat liver PI exchange proteins sustain a maximal rate of PI transfer from pancreatic microsomes to liposomes, approx. 50% of the PI is transferred with single-state kinetics and an exchange half-life of 5–6 min. Furthermore, the relative size of this exchangeable pool is independent of the level of exocrine secretory activity of the pancreatic tissue from which the microsomes are isolated. We thus find no evidence to suggest that the 33–40% net loss of PI content in pancreatic exocrine cells which occurs during cholinergic stimulation is primarily restricted to

either the exchangeable or non-exchangeable PI pool in pancreatic microsomes. However, it is possible that one of these two pools may be preferentially degraded during in vivo cholinergic stimulation, but that the activity of the phospholipid exchange proteins in the pancreatic exocrine cells tends to maintain a constant proportion of these two pools in the cellular membranes. Indeed, if we prepare post-microsomal supernatants from pancreatic tissue and subject them to ion-exchange chromatography as described in Materials and Methods, we can detect PI exchange activity whose specific activity (exchange activity/mg protein) appears to be independent of the level of exocrine secretory activity of the pancreatic tissue from which the post-microsomal supernatants are prepared (results not shown).

Finally, it should be noted that we have confirmed the finding by Brophy et al. [3] of a biphasic rate of protein-mediated PI transfer from rat liver microsomes and concur with their analysis that half of the exchangeable PI appears to have an exchange half-life of about 5 min and the other half an exchange half-life of about 60 min. The apparent exchange half-life of the rapidly exchangeable PI pool in liver microsomes thus closely matches that of the single exchangeable PI pool in pancreatic microsomes. If, as Brophy et al. have suggested, the rapidly and slowly exchangeable PI pools in liver microsomes represent free and protein-bound PI, respectively, residing in the outer phospholipid layer of the microsomal membranes, it then appears that pancreatic microsomes differ from liver microsomes in that (1) only 50–60% of the PI resides in the outer phospholipid layer and (2) almost all of the PI in the outer layer is not associated with membrane proteins.

Acknowledgment

This research was supported by NIH grant 1 R01 AM20865 to F.S.

References

1 Helmkamp, H.M., Harvey, M.S., Wirtz, K.W.A. and Van Deenen, L.L.M. (1974) J. Biol. Chem. 249, 6382–6389
2 Lumb, R.H., Kloosterman, A.D., Wirtz, K.W.A. and Van Deenen, L.L.M. (1976) Eur. J. Biochem. 69, 15–22
3 Brophy, P.J., Burbach, P., Nelemans, S.A., Westerman, J., Wirtz, K.W.A. and Van Deenen, L.L.M. (1978) Biochem. J. 174, 413–420
4 Michell, R.H. (1975) Biochim. Biophys. Acta 415, 81–147
5 Hokin, M.R. and Hokin, L.E. (1953) J. Biol. Chem. 203, 967–977
6 Clements, R.S. and Rhoten, W.B. (1976) J. Clin. Invest. 57, 684–691
7 Michell, R.H. and Jones, L.M. (1974) Biochem. J. 138, 47–52
8 Scott, T.W., Mills, S.C. and Freinkel, N. (1968) Biochem. J. 109, 325–332
9 Hokin, M.R., Hokin, L.E., Saffran, M., Schally, A.V. and Zimmerman, B.U. (1958) J. Biol. Chem. 233, 811–818
10 Hokin, L.E. and Huebner, D. (1967) J. Cell Biol. 33, 521–530
11 Hokin-Neaverson, M. (1974) Biochem. Biophys. Res. Commun. 58, 763–768
12 Farese, R.V., Larson, R.E. and Sabir, M.A. (1982) Biochim. Biophys. Acta 710, 391–399
13 Harris, D.W. and Hokin-Neaverson, M. (1977) Fed. Proc. 36, Abstr. 1941, 639
14 Rickwood, D. and Birnie, G.D. (1975) FEBS Lett. 50, 102–110
15 Morris, S.J. and Schovanka, I. (1977) Biochim. Biophys. Acta 464, 53–64
16 Slaby, F. and Farquhar, M.G. (1980) Mol. Cell Endocrinol. 18, 21–32
17 Yavin, E. and Zutra, A. (1977) Anal. Biochem. 80, 430–437
18 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275