Asymmetric synthesis of ethanolamine phospholipids in chicken brain microsomes, through the cytidine pathway

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1. INTRODUCTION

Phosphatidylcholine and phosphatidylethanolamine are the major phospholipids in the membranes of nervous tissue. Studies on their intramembrane localization showed that these phospholipids have an asymmetric distribution in microsomes [1] and in synaptosomal plasma membranes [2–3]. These observations raise the question on the mechanisms by which this asymmetry is produced. The de novo synthesis of phosphatidylcholine and phosphatidylethanolamine in brain is largely ensured by Kennedy's pathway [4]. The last step of their synthesis is catalyzed by phosphocholine transferase (EC 2.7.8.1) and phosphoethanolamine transferase (EC 2.7.8.2), respectively. Recent studies on the localization of these enzymes suggest that they are differentially embedded in the microsomal membrane [5]. Therefore, it seems likely that both enzymes may participate in the asymmetric assembly of phospholipids in the membranes. The synthesis of phosphatidylethanolamine and the corresponding plasmalogens by the cytidine pathway was therefore investigated, and the site of incorporation of the labelled precursors was determined. The results are the subject of this report.

Abbreviations: PE, phosphatidylethanolamine; LPE, lysophosphatidylethanolamine; TNBS, 2,4,6-trinitrobenzene sulfonic acid

2. MATERIALS AND METHODS

Microsomes were obtained from adult chicken brain as in [6] and incubated with CDP [^{14}C]ethanolamine as in [7]. The reaction was stopped by addition of Ca^{2+} (final conc. 1.25 mM).

The determination of the radioactivity of phosphatidylethanolamine and the ethanolamine plasmalogens in the outer and inner leaflet of the microsomal bilayer was determined using trinitobenzene sulfonic acid (TNBS) in non-permeating conditions. The labelled microsomes were incubated with 1.66 mM TNBS at 0°C for 20 min as in [8]. In these conditions ~32% of the total ethanolamine phospholipids reacted with TNBS. The reaction was terminated by the addition of perchloric acid (final conc. 0.8 N) and the microsomes centrifuged for 30 min at 5000 rev./min. The pellet was washed with 0.6 N perchloric acid, and the lipid extracted with 20 vol. chloroform–methanol (2:1, v/v) as in [9].

The treatment of the microsomes with perchloric acid hydrolysed the plasmalogens into lysophosphatidylethanolamine and thus radioactivity determined in the lysophosphatidylethanolamine and those which reacted with TNBS will represent the incorporation of the label into the plasmalogens situated in the inner and outer leaflet of the microsomal membrane.

The lipids were separated by thin-layer chromatography on silica-gel plates (Merck-Darmstadt) using chloroform–methanol–ammonia 27% (65:35:5, by vol.) as a development system, and

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visualized by exposure to iodine vapours. The phosphatidylethanolamine, the corresponding lyso-compounds reacting with TNBS (TNBSPE and TNBSLPE) and those which did not react with TNBS (PE and LPE) were scraped into a scintillation vial and counted in 0.5 ml water and 10 ml Rotiszint 22 in a scintillation spectrometer Intertechnique SL. 30. The amount of product synthesized was calculated from the specific activity of CDP-[^{14}C]ethanolamine measured in the same conditions.

3. RESULTS AND DISCUSSION

Fig.1 shows the distribution of the newly synthetized phosphatidylethanolamine in the two microsomal leaflets after incubation with CDP [^{14}C]ethanolamine. The amount of labelled PE in the outer leaflet (TNBS PE) was higher than in the inner leaflet (PE) and both increased over 0.5–60 min. However, the ratio of labelled PE reacting with TNBS over those not reacting with TNBS decreased from ~3.2–~2.0 over 0.5–10 min and remained constant thereafter.

Similar results were also obtained for the synthesis of ethanolamine plasmalogens (fig.2). The amount of labelled plasmalogens in the outer leaflet (TNBSLPE) was also higher than in the inner one (LPE). Similarly to the diacyl compounds, the amount of radioactive plasmalogens increased in both leaflets, while the ratio [^{14}C]TNBSLPE/[^{14}C]LPE decreased from ~2.6–~1.7 over 0.5–10 min and remained constant thereafter. These results indicate that the rate of increase of radioactive plasmalogens and diacyl compounds is higher in the inner than in the outer leaflet.

The much higher amount of newly formed PE and LPE present in the outer leaflet after short time of incubation may indicate that the diacylglycerophosphoethanolamine and the corresponding plasmalogen are synthesized on the external side of the microsomal vesicles which correspond to the cytoplasmic side. The increasing amount of radioactive ethanolamine phospho-

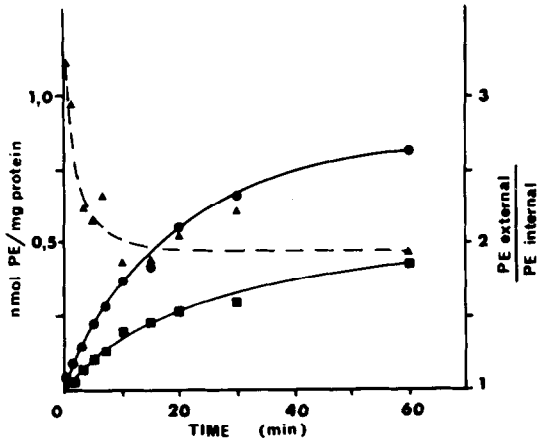

Fig.1. Microsomes (1.75 mg protein) were incubated with CDP [^{14}C]ethanolamine (1.25 mM) spec. act. 2.87 × 10^6 cpm/μmol in 170 mM $NaHCO_3$ (pH 8.00), 10 mM $MnCl_2$ in 0.400 ml final vol. The reaction was stopped by 1.25 mM Ca^{2+} and the microsomes treated with TNBS (1.66 mM) at 0°C for 20 min as in [8]. Phospholipids were extracted and separated as in section 2. Of the total PE, 34.9% reacted with TNBS. The results, expressed as nmol/mg protein as function of incubation time with CDP [^{14}C]ethanolamine, are an average of 3 expt: (•—•) [^{14}C]phosphatidylethanolamine in the outer leaflet (TNBSPE); (▪—▪) [^{14}C]phosphatidylethanolamine in the inner leaflet (PE); (▲ - - ▲) ratio of newly synthesized PE: [^{14}C]PE external/[^{14}C]PE internal.

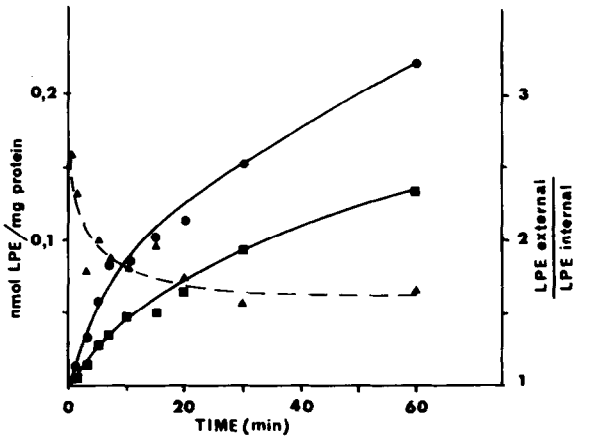

Fig.2. Microsomes were incubated as in fig.1. Of the total LPE (ethanolamine plasmalogens), 30.3% reacted with TNBS. The results, expressed as in fig.1, are an average of 3 expt.: (•—•) [^{14}C]ethanolamine plasmalogens in the outer leaflet (TNBSLPE); (▪—▪) [^{14}C]ethanolamine plasmalogens in the inner leaflet (LPE); (▲ - - ▲) ratio of the newly synthesized LPE: [^{14}C]LPE external/[^{14}C]LPE internal.

lipids in the inner leaflet suggests a transfer of these molecules from the outer to the inner leaflet. This transfer seems to be very rapid since the ratios [^{14}C]TNBSPE/[^{14}C]PE and [^{14}C]TNBSLPE/[^{14}C]-LPE decrease very rapidly and become constant after ~ 10 min incubation.

The mechanism which controls this transfer is unknown. It may be that either a certain amount of the synthesized molecules of ethanolamine phospholipids are discharged by the phosphoethanolamine transferase into the inner leaflet or that the enzyme discharges all molecules into the outer leaflet where another mechanism ensures the passage to the inner leaflet. The transmembrane position of the phosphoethanolamine transferase in brain microsomes suggested in [5] may be consistent with the first hypothesis.

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REFERENCES

[1] Freysz, L., Dreyfus, H., Vincendon, G., Binaglia, L., Roberti, R. and Porcellati, G. (1982) in: Phospholipids in the Nervous System: vol. I, Metabolism (Hovrocks L.A. et al. eds) Raven Press, New York, in press.

[2] Fontaine, R.N., Harris, R.A. and Schroeder, F. (1980) J. Neurochem. 34, 269–277.

[3] Smith, A.P. and Loh, H.H. (1976) Proc. West Pharmacol. Soc. 19, 147–151.

[4] Ansell, G.B. (1973) in: Form and Function of Phospholipids (Ansell, G.B. et al. eds) pp. 377–422, Elsevier Biomedical, Amsterdam, New York.

[5] Freysz, L., Harth, S. and Dreyfus, H. (1982) J. Neurochem. 38, 582–587.

[6] Porcellati, G., Arienti, G., Pirotta, M.G. and Giorgini, D. (1971) J. Neurochem. 18, 1395–1417.

[7] Freysz, L., Horrocks, L.A. and Mandel, P. (1977) Biochim. Biophys. Acta 489, 431–439.

[8] Rothman, J.E. and Kennedy, E.P. (1977) J. Mol. Biol. 110, 603–618.

[9] Folch-Pi, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.