LACK OF COMPETITION BETWEEN PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE SYNTHESIS IN THE MEMBRANES OF *ENTODINIUM CAUDATUM*

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1. Introduction

Recent evidence indicates that in higher plants, phosphatidylethanolamine synthesis from CDP-ethanolamine and phosphatidylcholine synthesis from CDP-choline are catalysed by a common enzyme. Thus Macher and Mudd [1] showed, using spinach microsomes, that CDP-choline was a powerful inhibitor of ethanolamine phosphotransferase (EC 2.7.8.1) while similarily CDP-ethanolamine inhibited choline phosphotransferase (EC 2.7.8.2). This they attribute to a common active centre, a conclusion which they strengthen by showing an identical behaviour of the activities toward thiol inhibitors and lyophilization. Studies on the kinetics of CDP-choline inhibition of ethanolamine phosphotransferase in castor bean endosperm have also suggested that a single enzyme utilizes both nucleotide substrates [2].

The situation concerning the synthesis of these two phospholipids in mammalian cells is less clear. During early work it was observed that the enzyme synthesizing phosphatidylcholine was more stable to lyophilization than that synthesizing phosphatidylethanolamine leading to the suggestion that two separate enzymes were involved [3]. This is supported by the susceptibility of cholinephosphotransferase to acylCoA inhibition compared with ethanolamine phosphotransferase [4] and the markedly different ratios between the two enzyme activities in various rat tissues [5,6]. Nevertheless CDP-choline does act as an inhibitor of ethanolamine phosphotransferase contained in a microsomal preparation from brain [6]. In addition the choline/ethanolamine phosphotransferase of rat liver has low specificity, transferring a variety of phosphorylated bases from their CDP-derivatives to diglyceride [7].

During recent studies in which we have been investigating relationships between macromolecule synthesis during membrane turnover in the anaerobic protozoon *Entodinium caudatum* we have attempted to change the rate of synthesis of individual macromolecules by specific methods which would not directly affect the synthesis of other macromolecules. Thus we have markedly varied the rate of synthesis of phosphatidylcholine by altering the CDP-choline concentration without in any way affecting the synthesis of phosphatidylethanolamine. This argues strongly against a single choline (ethanolamine) phosphotransferase operating in the organism.

2. Materials and methods

Growth of the *E. caudatum* and the preparation of the membrane fraction has been described previously [8,9]. CDP-[*Me*-^{14}C] choline and CDP-[2-^{14}C] ethanolamine were biosynthesized and separated as described in these papers. CDP-choline was obtained from Sigma (St. Louis, USA). A total membrane fraction (equivalent to 0.1 ml packed protozoal cells) was incubated in a medium containing 7.7 mM $MgSO_4$, 77 mM Tris-buffer (pH 8.3) 4 mM EGTA and suitable aliquots of ^{14}C-labelled CDP-choline or CDP-ethanolamine (25 000 dpm). Incubation was for 1 h at 37°C under N_2/CO_2 19:1, v/v. Incorporation was stopped by adding 4 ml chloroform/methanol 2:1, v/v and shaking vigorously. After centrifuging the lower phase was washed four

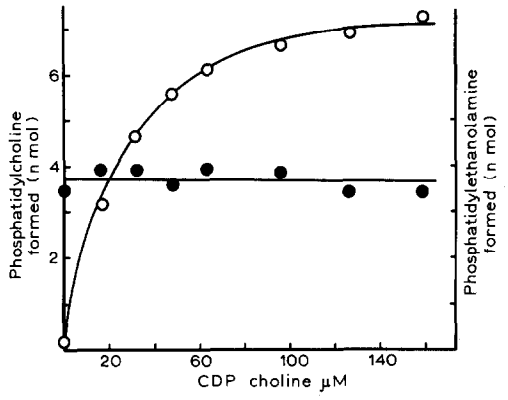

Fig.1. The effect of CDP-choline concentration on the synthesis of phosphatidylcholine (○) and phosphatidylethanolamine (●) from radioactive CDP-choline and CDP-ethanolamine respectively.

times with 2 vol. chloroform/methanol/H_2O 3:45:49 v/v/v. An aliquot of the lower phase was taken for liquid scintillation counting. Preliminary experiments showed that phosphatidylcholine and phosphatidylethanolamine were the only phospholipids labelled from radioactive CDP-choline and CDP-ethanolamine respectively.

3. Results

Figure 1 records the rate of synthesis of phosphatidylcholine as a function of CDP-choline concentration showing a typical Michaelis-Menton relationship. In this and an entirely separate experiment a K_m value of 19 μM CDP-choline was obtained.

On the same figure is plotted the effect of the same concentration-range of CDP-choline on the synthesis of phosphatidylethanolamine from CDP-[2-^{14}C]-ethanolamine showing constancy of incorporation.

4. Discussion

With the present membrane preparation, the ethanolamine phosphotransferase activity seems to be independent of CDP-choline addition over a range which causes a corresponding increase in choline phosphotransferase activity of over five-hundred-fold and which results in substrate saturation of the later enzyme. This indicates that, in *E. caudatum*, phosphatidylcholine and phosphatidylethanolamine are synthesized by independent enzymes. It has already been shown that in the same organism choline kinase and ethanolamine kinase have separate identities [10] suggesting entirely separate pathways for the biosynthesis of these two phospolipids.

In the present studies, diglyceride has not been added to the enzyme incubation, and the synthesis has relied on intrinsic diglyceride present in the membrane preparation. It was felt that the introduction of diglyceride dispersed with detergent might disrupt membrane assembly or turnover. It has been shown that diglyceride addition did not enhance choline phosphotransferase activity in *E. caudatum* membranes [8,9], although this was not tested at the high substrate concentrations of CDP-choline used in the present investigation. However, since these high CDP-choline concentrations caused no inhibition of ethanolamine phosphotransferase it is very unlikely that the latter enzyme could be competing with choline phosphotransferase for limited supplies of diglyceride. In experiments using plant microsomes, showing inhibition of ethanolaminephosphokinase by CDP-choline [1,2], diglyceride was not added, whereas with the much smaller inhibitions observed using brain microsomes [6], diglyceride dispersed with Tween-20 had been added.

Since phosphatidylcholine and phosphatidylethanolamine are the major structural phospholipids present in *E. caudatum* membranes [11] it seemed possible that a major change in the rate of synthesis of one might reflect on the synthesis of the other, irrespective or not as to whether the enzymes synthesizing them have separate identities. Thus if new lipoprotein membranes units were formed as distinct entities during membrane replacement or growth one might expect that a dramatic change in the synthesis of one component might have some feedback control over the other. The present results suggest this is not so and it seems likely therefore that the constancy of membrane phospholipid composition is achieved by other means.

The same conclusion has now been drawn for the relationship between protein and phospholipid synthesis in a variety of experimental systems. The suppression of protein synthesis in liver membranes by inhibitors shows that it and phospholipid synthesis are not tightly coupled [12] and in *Escherichia coli* the blockage of phosphoglyceride synthesis does not

prevent the synthesis and integration of proteins into membranes [13].

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