Substrate selectivity of acyl-CoA:lysolecithin acyltransferase from rabbit lung

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Summary

The influence of both polar head and acyl chain of lysophospholipid on the activity of partially purified acyl-CoA:lysolecithin acyltransferase from rabbit lung was studied. It was concluded that the presence of methyl groups on the nitrogen of the base was essential for recognition of lysophospholipid as substrate by the enzyme. With respect to the acyl chain length and saturation, the activity followed the order:

16:0 = 18:1 > 14:0 > > > 18:0 = 12:0.

Also, the effect on the activity of the acyl chain on acyl-CoA was studied. The activity showed great selectivity for saturated acyl-CoAs. The activity with polyunsaturated fatty acids was very low and in the case of arachidonoyl-CoA was almost negligible.

The comparison between crude microsomal preparations and partially purified preparations allowed to suggest that it could exist two different acyl-CoA:lysolecithin acyltransferases differing in their selectivity towards saturated and unsaturated fatty acids.

Abbreviations

LPC, lysophosphatidylcholine. LPDME, lysophosphatidyl-dimethylethanolamine. LPE, lysophosphatidylethanolamine. PC, phosphatidylcholine. PDME, phosphatidyl-dimethylethanolamine. PE, phosphatidylethanolamine.

Fatty acids: 12:0, lauric; 14:0, miristic; 16:0, palmitic; 18:0, stearic; 18:1, oleic; 18:2, linoleic and 20:4, arachidonic.

Introduction

To prevent alveolar collapse and plasma transudation, the alveolar surface is lined with a material which has surface properties and is known as pulmonary surfactant. 1,2-dipalmitoyl-sn-glycero-3-phosphocholine is the major component of surfactant from mammalian lung tissue and is considered to play the main role in determining the properties of pulmonary surfactant (1–3).

The mechanism of biosynthesis of this disaturated phospholipid is still an open question. The CDP-choline pathway, principal route for the biosynthesis of phosphatidylcholine in lung (4–6), yields mainly sn-2 unsaturated species; therefore, additional mechanisms are necessary to explain the high levels of palmitic acid on the sn-2 position of lung phosphatidylcholine.

Two pathways have been described in lung tissue that can account for the remodelling of phosphatidylcholines. Both pathways start with the hydrolysis of sn-2 unsaturated species by the action
of phospholipase A₂ (7), yielding lysophosphatidylcholine.

Akino et al. (8) proposed that the soluble fraction enzyme lysolecithin:lysolecithin acyltransferase (EC 3.1.1.5), which catalyzes transesterification between two molecules of lysolecithin, could play an important role in the synthesis of dipalmitoylphosphatidylcholine. Recently, this enzyme has been found in rabbit lung and purified (9); also, some aspects of its conformation, activity and specificity have been studied (10–12).

In the microsomal fraction there exists another enzyme, acyl-CoA:lysolecithin acyltransferase (EC 2.3.1.23), which catalyzes the direct reacylation of lysophosphatidylcholine with acyl-CoA. This enzyme is responsible for the second remodelling mechanism described in the literature (13–15). Nevertheless, acyl-CoA:lysolecithin acyltransferase has been reported to exhibit a higher specificity for unsaturated acyl-CoAs in vitro (16). Also, radioactive lysolecithin is known to be acylated preferentially with diene and tetraene fatty acids in vivo and in slices (8). In contrast with these results, radio-labelled palmitate is mainly incorporated in the sn-2 position when added to lung slices, suggesting the involvement of acyl-CoA:lysolecithin acyltransferase in the formation of disaturated species of phosphatidylcholine (8, 17).

It is difficult at the present to evaluate the relative contribution of this enzyme in the synthesis of dipalmitoylphosphatidylcholine, because of the considerable variation in the specificities reported for acyl-CoA in lung (4, 16, 18, 19).

The present paper deals with the study of the influence of both the nature of polar head and acyl chain of substrate lysophospholipid on the activity of acyl-CoA:lysolecithin acyltransferase from rabbit lung. Also, the specificity for the acyl-CoA donor is presented. The experiments were carried out in a purified microsomal membrane preparation which was substantially free from acyl-CoA hydrolase contamination (15). The absence of this hydrolytic activity is important because crude microsomal preparations show high hydrolytic activity which can be responsible for the different specificities reported in the literature for acyl-CoA:lysolecithin acyltransferase.

### Materials and methods

#### Materials

1-[14C]Palmitoyl-sn-glycero-3-phosphocholine, 1-[14C]palmitoyl-CoA and 1-[14C]oleoyl-CoA (sp. act. 59, 57 and 56 μCi/μmol respectively) were purchased from The Radiochemical Center (Amersham, UK). Dipalmitoylphosphatidyl ethanolamine, dipalmitoylphosphatidyl dimethyl ethanolamine, 1,2-diacylphosphatidylcholines (acyl: 12:0, 14:0, 16:0, 18:0 and 18:1), acyl-CoA thioesters (acyl: 16:0, 18:0, 18:1, 18:2 and 20:4), sodium deoxycholate, Tris base and phospholipase A₂ (Crotalus adamanteus) were from Sigma (St. Louis, USA). All other chemicals and solvents were analytical reagent grade from Merck (Darmstadt, FRG). Brij 35 was a generous gift from ICI España SA.

#### Enzyme preparation

Acyl-CoA:lysolecithin acyltransferase was partially purified from rabbit lung microsomes as previously described (15) by a procedure which involves treatment of microsomes with detergents (sodium deoxycholate and Brij 35).

#### Substrates

Nonlabelled lysophosphatidylcholines with 12:0, 14:0, 16:0, 18:0 and 18:1 fatty acids on the sn-1 position, 1-palmitoylphosphatidylethanolamine and 1-palmitoylphosphatidyl dimethylethanolamine were prepared by phospholipase A₂ degradation of the corresponding diacyl derivatives as previously described (11).

The mixture of lysophospholipids and 1-palmitoyllysolecithin was prepared by mixing the corresponding aliquots of lyso compound dissolved in chloroform/methanol (2:1, v/v) and 50 nmol of 1-palmitoyllysolecithin in the same solvent. After nitrogen evaporation, the mixture was suspended in 0.1 M Tris buffer, pH 7.4 and sonicated at 0°C and 16 microns in a MSE sonicator until the solution was clear.

The concentration of the mixtures are expressed in the legends of Figures.