COENZYME A-DEPENDENT ESTERIFICATION OF CHOLESTEROL IN RAT LUNG

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1. Introduction

Lung contains free and esterified cholesterol [1–3]. The important physiological substance surfactant, contains free and esterified cholesterol [2,3] and this substance is necessary for the respiratory properties of lung operating at 37°C [4]. Lungs have the ability to synthetise cholesterol [5–7] and in addition, lung can take up cholesterol from plasma lipoproteins [8]. A portion of the newly synthesised cholesterol in lung is rapidly esterified [7] and the esterification of cholesterol in lung has been demonstrated indirectly [9]. This report presents evidence for a coenzyme A-dependent enzymic system involved in the esterification of cholesterol in lung.

2. Materials and methods

2.1. *Chemical and radiochemical products*

[4-^{14}C]Cholesterol (>50 Ci/mol) was obtained from the Radiochemical Centre (Amersham). Adenosine 5′-triphosphate (ATP) and coenzyme A were purchased from Boehringer Mannheim GmbH (Mannheim). Glutathione (reduced form) and bovine serum albumin (free of fatty acids) were obtained from Sigma (London).

2.2. *Tissue fractionation procedure*

Male Wistar rats of an inbred strain (~200 g body wt) were used in these studies. The animals were anaesthetised with pentobarbital and blood was drawn by aortic puncture. The lungs were washed with isotonic saline to remove most of the blood and the lung tissue was then homogenised in 0.33 M sucrose containing 0.01 M potassium phosphate buffer (pH 7.4). The cell debris, mitochondria, microsomes and lamellar bodies were prepared as in [10]. This method employed a discontinuous sucrose gradient technique and was used in some initial studies to identify the location of the cholesterol esterification system. However as the subcellular fractionation method is time consuming and results in a loss of enzymic activity a classical microsomal preparation method was used for most of the enzyme studies.

The lung tissue was finely minced with scissors and then homogenised in 4 vol. potassium phosphate buffer 0.1 M (pH 7.4) in a glass–Teflon homogeniser employing 4 slow passes of the plunger followed by 4 fast passes. This homogenate was centrifuged at 8000 × *g* for 20 min, the pellet was discarded and the supernatant was centrifuged at 105 000 × *g* for 1 h. The resultant microsomal pellet was resuspended in potassium phosphate buffer 0.1 M (pH 7.4) containing 1 mM EDTA. The resuspended microsomal preparation was centrifuged once more at 105 000 × *g* for 1 h. This washed microsomal preparation was resuspended in potassium phosphate buffer 0.1 M (pH 7.1) and this suspension was employed at the source of the enzyme under study.

2.3. *Enzyme assay*

The enzyme assay system employed was modified from the procedure in [11]. The microsomal suspension (50 μl equivalent to 0.4 mg protein) was incubated in a potassium phosphate buffer (0.1 M) pH 7.1, ATP (4 mM), coenzyme A (0.5 mM), $MgCl_2$ (4 mM), bovine serum albumin free from fatty acids (1.5 mg) and glutathione (3.3 mM). The final volume of the reaction mixture was 3 ml. Incubations were at 37°C in a shaking water bath at 90 osc./min in air. At the start of each incubation [4-^{14}C]cholesterol (0.05 μCi in 50 μl acetone) was added.

The incubation was stopped by the addition of 5 ml methanol and the resultant mixture boiled for a

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few minutes to denature and precipitate the proteins. The aqueous methanolic solution was cooled and 10 ml chloroform added to each mixture. Thereafter the lipid content of each incubation was extracted according to a standard lipid extraction method [12]. Free and esterified cholesterol were separated by thin-layer chromatography using silica-gel H plates and a petroleum ether:diethyl ether:acetic acid (70:30:1) solvent system. The bands of free and esterified cholesterol were located by radioactive scanning in a Panax thin-layer radioactive scanner. The radioactive bands were scraped into counting vials and the radioactivity measured by liquid scintillation using a toluene-based scintillator and employing a Packard liquid scintillation spectrometer. The types of newly synthesised labelled cholesterol esters were determined by thin-layer chromatography using silica-gel H plates impregnated with $AgNO_3$, using benzene:hexane (1:1) as the solvent [11]. The composition of the cholesterol esters of rat lung microsomes was determined after hydrolysis of the ester extraction, methylation of the fatty acids followed by gas–liquid chromatography [13].

The protein content of the incubation mixture was measured [14] and cholesterol was determined as in [15].

3. Results

In this biological system, cholesterol may be esterified with fatty acids at pH 5 without addition of cofactors to the incubation mixture. At pH 7 however the esterification of cholesterol requires coenzyme A and ATP. The subcellular localisation of the coenzyme A-dependent esterification system is shown in table 1.

The esterification of cholesterol in the microsomal fraction has been shown to be coenzyme A-dependent and the optimum pH for this esterification reaction is shown in fig.1.

Dependence of the reaction on the protein content is shown in fig.2 while the cofactor requirement of this microsomal esterification system is shown in table 2. It should be noted that the esterification reaction exhibits a coenzyme A saturation curve in which maximal esterification activity occurs at ~0.3 mM coenzyme A.

The time course of the esterification reaction is illustrated in fig.3, while the different types of cholesterol ester formed is shown in table 3.

Since it is known that feeding cholesterol to rats results in absorption of the sterol and deposition of esterified cholesterol in various tissues a study was made of the effect of feeding cholesterol on the cholesterol esterification reaction in lung (table 4).

4. Discussion

Here we show that there is a coenzyme A-dependent enzymic reaction in lung capable of effecting the esterification of cholesterol. The characteristics of this enzymic reaction are similar to the acyl-coen-

Table 1
Cholesterol content and subcellular distribution of coenzyme A-dependent esterification of cholesterol in rat lung

	Cholesterol content (μg/mg prot.)		% [4-^{14}C]Cholesterol esterified	
	Free	Esterified	1st Expt	2nd Expt
Mitochondria + lysosome fraction	40.1 ± 1.5[c]	7.7 ± 1.7	0.65 ± 0.04[a]	0.71 ± 0.09
Microsomes	61.1 ± 4.7	6.4 ± 0.6	1.10 ± 0.05	1.21 ± 0.14
Lamellar bodies	113.0 ± 1.0	36.4 ± 1.1	0.04 ± 0.02	0.08 ± 0.01
Cytosol[b] (105 000 × *g* supern.)	1.7 ± 0.1	3.3 ± 0.7	–	–

[a] ± SEM, triplicate experiment; [b] no activity detected; [c] ± SEM, 4–6 determinations

Each 3 ml incubation contained 0.4 mg protein, cofactors and [4-^{14}C]cholesterol as in section 2; they were conducted for 3 h

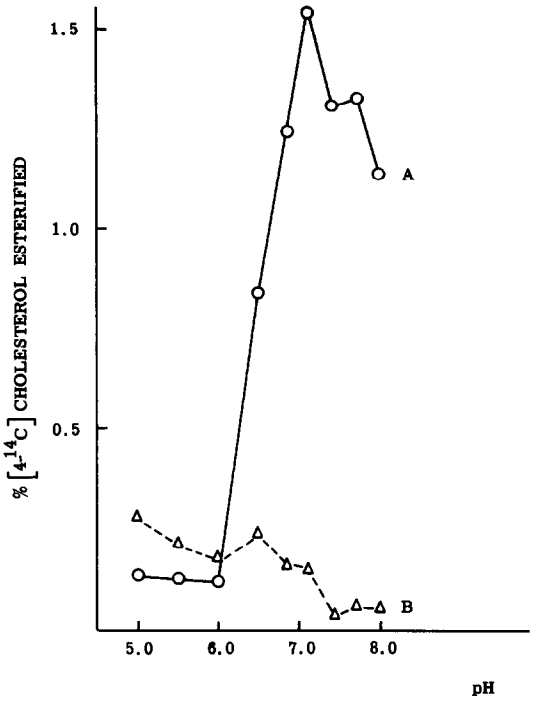

Fig.1. Effect of pH on cholesterol esterification by rat lung microsomes. The pH range was obtained with potassium phosphate buffer 0.1 M. The incubation conditions were as in section 2 except for coenzyme A which was 0.5 mM in (A) and nil in (B).

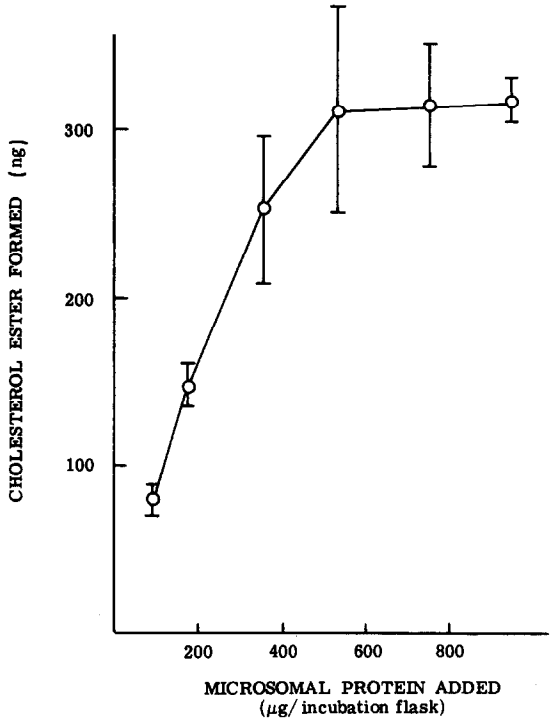

Fig.2. Effect of microsomal protein content of the incubations on the amounts of cholesterol esters formed. Each incubation was for 3 h and contained all cofactors and [4-^{14}C]cholesterol as in section 2. Each point represents the mean (± SEM) of 3 different incubations.

zyme A–cholesterol acyl transferase reaction (ACAT) observed in liver [11], and the similar reaction observed in arteries [16]. However, one of the differences between the liver ACAT reaction and the reaction described in lung is that in the absence of ATP the liver ACAT reaction does not occur, whereas in lung there is some esterification of cholesterol in the absence of ATP. The other difference between the

Table 2
Cofactor requirement for cholesterol esterification in rat lung microsomes

	% [4-^{14}C]Cholesterol esterified	
	Expt 1	Expt 2
Complete system	1.28 ± 0.02[a]	3.07 ± 0.07
ATP omitted	0.68 ± 0.01	1.69 ± 0.08
Coenzyme A omitted	0.18 ± 0.01	0.59 ± 0.04
ATP and coenzyme A omitted	0.18 ± 0.03	0.18 ± 0.02
Control (boiled microsomes)	0.18 ± 0.01	0.22 ± 0.04

[a] SEM, 3 determinations

The incubations were for 3 h in expt 1 and 6 h in expt 2; conditions as in section 2

Table 3
Cholesterol esters composition and distribution of cholesterol esters synthesised by rat lung microsomes

Fatty acids	Percentage composition of cholesterol esters	Percentage [4-^{14}C]cholesterol esterified to different classes of fatty acids	
16:0	32.2 ± 1.8[a]		
18:0	13.0 ± 1.9	Saturated	24.3 ± 0.7
16:1	5.6 ± 0.3		
18:1	24.3 ± 1.9	Monounsaturated	16.7 ± 0.8
18:2	11.6 ± 0.5	Diunsaturated	10.0 ± 0.9
18:3	2.5 ± 0.9	Triunsaturated	2.6 ± 2.0
20:1	3.0 ± 1.3		
20:4	7.7 ± 0.7	Polyunsaturated	46.3 ± 1.7

[a] SEM, 6 determinations

The incubations were for 3 hours; conditions as in section 2

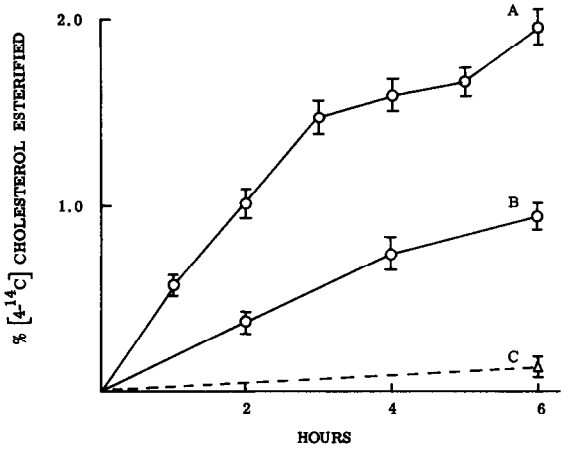

Fig.3. Time course of cholesterol esterification in rat lung microsomes. Each 3 ml incubation contains 0.4 mg microsomal protein [4-^{14}C]cholesterol and all cofactors as in section 2. In (A) coenzyme A was 0.5 mM, in (B) 0.05 mM and in (C) no coenzyme A was added to the incubations. Each point represents the mean (± SEM) of 3 different incubations.

ACAT reaction and the reaction described in lung is that the ACAT reaction in liver and in arteries appears to occur optimally with monounsaturated fatty acyl CoAs whereas in lung the esterification of cholesterol under these enzymic conditions occurs first with polyunsaturated fatty acids and then with saturated fatty acids. This result which is in contrast with the fatty acid distribution of the microsomal cholesteryl esters suggests a faster turnover for cholesteryl arachidonate in lungs. From the calculated activities in these studies (1.9 nmol . h^{-1} . mg $protein^{-1}$) and by comparing these results with published work it seems that the activity in liver is greater than that in lungs and that lung tissue has an activity greater than that observed in arteries. The esterification of cholesterol in arteries may proceed by the reversal of the cholesterol esterase enzyme with an acidic pH optimum. Such an esterification reaction in arteries may proceed without cofactors [15]. Here it has been difficult to attribute the enzymic activity to any special cell type in lung since there are ~40 different cell species in this tissue [17]. These cell types include cells in blood vessels, capillaries, epithelial cells and the specialised cells which produce lung surfactant [17]. The activity of this cholesterol-esterifying reaction may not be related to alveolar macrophage activity since there is no ACAT activity in alveolar macrophages [18]. In these studies cholesterol feeding did not produce a marked change in the activity of this cholesterol esterifying system. However, this

Table 4
Effect of cholesterol feeding on cholesterol esterification in rat lung

Cholesterol feeding duration	20 days	30 days
Control rats	1.19 ± 0.05[a]	1.17 ± 0.03
Cholesterol fed rats	1.39 ± 0.11	1.23 ± 0.08

[a] Mean ± SEM, 6 determinations

Rats were fed a diet containing 1% cholesterol. Incubations were for 2 h; conditions as in section 2. Results are expressed as % [4-^{14}C]cholesterol esterified

could be due to the fact that the rat has considerable resistance to cholesterol feeding and tends to retain most of the dietary cholesterol load as esterified cholesterol in liver.

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