PRESENCE OF CHOLESTEROL ESTER SYNTHETASE ACTIVITY IN GUINEA PIG GALLBLADDER EPITHELIUM

M. T. Ravi Subbiah and Betty A. Dicke

Cardiovascular Research Unit
Mayo Clinic and Mayo Foundation
Rochester, Minnesota 55901

Received December 2,1976

SUMMARY: This study is the first to demonstrate the presence of cholesteryl ester synthetase activity in the gallbladder epithelium. Using epithelium of the guinea pig gallbladder, the study demonstrated that the enzyme was localized mainly in the particulate fraction. The enzyme required CoA and ATP for activity and displayed a pH optimum of 7.0. The uptake of biliary cholesterol by gallbladder (shown by other investigators) and the presence of cholesteryl ester synthetase activity (demonstrated in this study) suggest that the gallbladder epithelium has an active role which might be important in conditions of cholesterol supersaturation in bile.

The gallbladder epithelium absorbs a number of substances (1), among which are oleic acid and lecithin (2,3). Neiderhiser et al. (4) showed that biliary cholesterol also can be absorbed by the gallbladder epithelium. These observations can be important in regard to gallstone formation when bile is supersaturated with cholesterol in relation to bile acids and lecithin, the two other biliary components that keep cholesterol in solution (5). Neiderhiser et al. (4) found that the instillation of [^{14}C]cholesterol into the in situ guinea pig gallbladder resulted in radioactivity in the cholesteryl ester fraction. This observation suggests that a cholesteryl esterifying enzyme, cholesteryl ester synthetase, is present within the gallbladder epithelium. Although cholesteryl ester synthetase is present in a number of animal tissues (6), its presence in the gallbladder has not previously been reported. This communication demonstrates for the first time the presence of a cholesteryl ester synthetase activity in gallbladder epithelium of the guinea pig and describes the properties of this enzyme.

METHODS

[4-^{14}C]Cholesterol (specific activity 50 mCi/mmol), [1-^{14}C]oleic acid (specific activity 50.6 mCi/mmol), [1-^{14}C]palmitic acid (specific activity 10

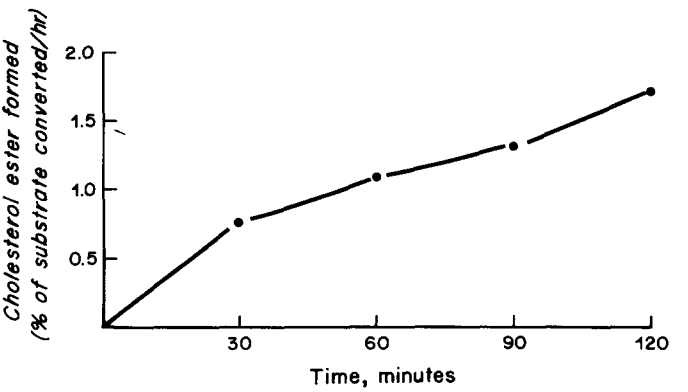

Fig. 1. Time course of cholesteryl ester synthetase activity in guinea pig gallbladder. Enzyme activity is expressed as percentage of added cholesterol converted to cholesteryl esters per mg protein in 1 hour.

---

mCi/mmol), and [1-^{14}C]linoleic acid (specific activity 40 to 60 mCi/mmol) were purchased from New England Nuclear (Chicago, IL) and checked for purity by thin-layer chromatography (7) using the solvent system of heptane:isopropyl ether:acetic acid (65:40:4, vol/vol/vol).

Guinea pigs (Hartley strain) weighing 600 to 800 g were maintained on regular guinea pig chow during the study. The guinea pigs were sacrificed, and their gallbladders were quickly excised, opened, and rinsed completely free of bile with saline.

The method used to measure the activity of cholesteryl ester synthetase was that of Goodman et al. (8) with slight modification (9). The tissue was homogenized in 0.1 M phosphate buffer (pH 7.4) for 1 minute. The homogenate was centrifuged at 1,000 x g for 20 minutes to remove the cell debris, and the 1,000 x g supernatant was used for most studies. When further fractionation of the homogenates was desired, the supernatant was centrifuged at 12,000 x g for 15 minutes to remove the mitochondrial fraction. This supernatant was then centrifuged at 100,000 x g for 30 minutes using an ultracentrifuge (Beckman LK-75). The pelleted microsomes and mitochondrial fraction were suspended in 0.1 M phosphate buffer to give an approximate protein concentration of 0.5 to 1 mg/ml. Aliquots of the microsomal, mitochondrial, and supernatant fraction were then used for the enzyme studies. The protein concentrations were measured by the method of Lowry et al. (10).

The incubation consisted of 0.5 to 1 mg of the 1,000 x g supernatant fluid or appropriate cell fraction in phosphate buffer (pH 7.4), ATP 15 μmol, CoA 15 μmol, NaF 15 μmol, $MgCl_2$ 12 μmol, and [^{14}C]cholesterol 10 nmol in 25 μl of acetone. The incubation was carried out for 1 hour at 37°C. At the end of incubation, 20 ml of chloroform:methanol (2:1, vol/vol) were added to stop the reaction; 50 to 100 μg of the carrier cholesteryl oleate and cholesterol were then added to each tube. The organic layer was removed, evaporated to a small volume, and subjected to thin-layer chromatography on silica gel G using the solvent system of heptane:isopropyl ether:acetic acid (65:40:4, vol/vol/vol) to separate free and esterified cholesterol (7). The bands corresponding to the free and esterified sterols were then scraped from the

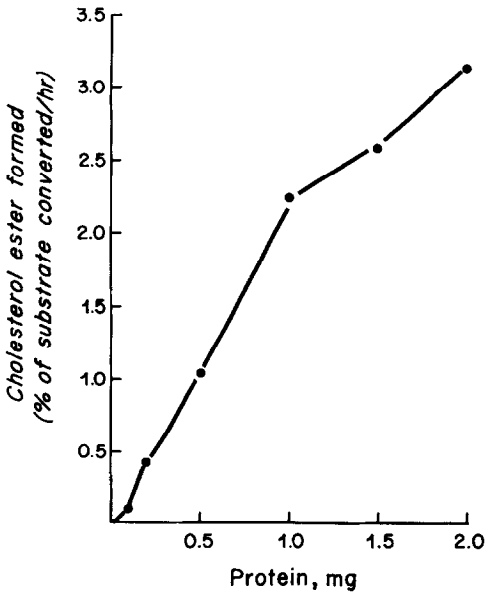

Fig. 2. Effect of protein concentration on activity of cholesteryl ester synthetase in guinea pig gallbladder. Enzyme activity is expressed as percentage of added cholesterol converted to cholesteryl esters per mg protein in 1 hour.

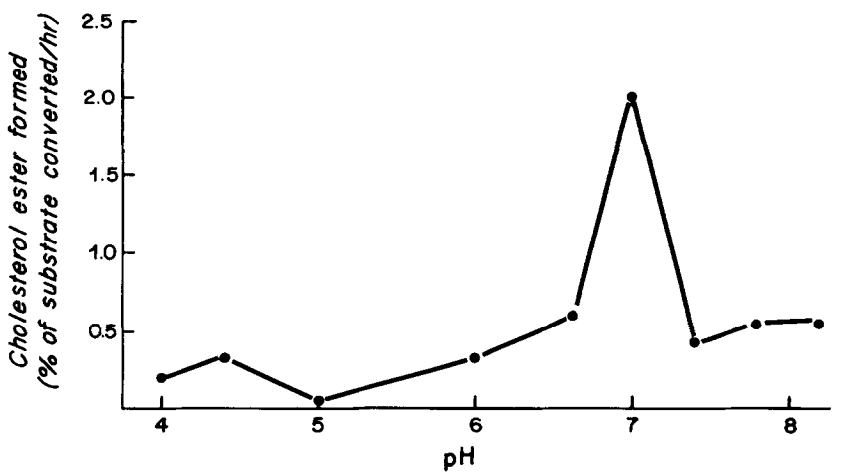

Fig. 3. Effect of pH on activity of cholesteryl ester synthetase in guinea pig gallbladder. Enzyme activity is expressed as percentage of added cholesterol converted to cholesteryl esters per mg protein in 1 hour.

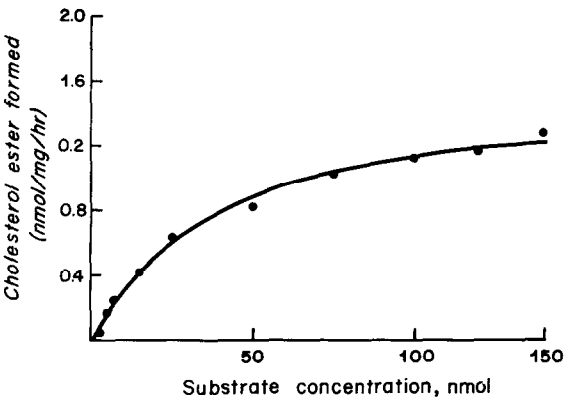

Fig. 4. Effect of substrate concentration on activity of cholesteryl ester synthetase in guinea pig gallbladder. Enzyme activity is expressed as nmol of product formed per mg protein in 1 hour.

Table 1. Effect of CoA and ATP on Cholesteryl Ester Synthetase Activity

Incubation condition	% Activity*
Complete system†	100.0
Complete system minus CoA	11.3
Complete system minus ATP	1.7
Complete system minus CoA and ATP	1.8

*Enzyme activity in complete system is assumed to be 100%.
†Complete system contained 2,000 x g supernatant fluid equivalent to 1 mg protein in phosphate buffer pH 7.0, ATP 15 μmol, CoA 15 μmol, NaF 15 μmol, $MgCl_2$ 12 μmol, and [^{14}C]cholesterol 10 nmol (500,000 dpm).

plate and eluted four times with 4 ml of chloroform. The extracts were dried and assayed for radioactivity in a liquid scintillation counter (Packard Model 3385). The mass of sterols and fatty acids was determined by gas-liquid chromatography as described previously (7,11).

RESULTS

The enzyme activity was fairly linear over a period of 2 hours, as seen in Figure 1. The nonenzymatic conversion was less than 0.05%, as indicated in the blank experiments using boiled enzymes. The enzyme activity was linear up to the protein concentration of 2 mg (Fig. 2). The maximal activity of the

Table 2. Subcellular Localization of Cholesteryl Ester Synthetase Activity

Cell fraction	% Activity*
Mitochondria	37.6
Microsomes	43.6
Supernatant	18.8

*Enzyme activity in each fraction is expressed as the percentage of the sum of the total activity in all three fractions. Incubation condition as in Table 1, except that 0.5 mg equivalent of each fraction was used.

Table 3. Fatty Acid Specificity of Cholesteryl Ester Synthetase Activity

Fatty acid substrate	Enzyme activity (M ± SE), pmol/mg/h*
Palmitic acid	13.3 ± 1.6
Oleic acid	11.7 ± 0.8
Linoleic acid	3.1 ± 0.3

*Each value represents the mean of 3 or 4 experiments. Incubation conditions as in Table 1.

enzyme was observed at a pH of 7.0 (Fig. 3). The enzyme activity showed a typical Michealis-Menton kinetics with regard to substrate saturation (Fig. 4). The apparent K_m value under the conditions described was about 41.3 nmol.

The optimal enzyme activity required CoA and ATP (Table 1). In the absence of CoA, a decrease of 88.7% in enzyme activity was observed, whereas in the absence of ATP, the decrease was 98.3%. The bulk of the subcellular localization of the enzyme was associated with the particulate fraction (43.6% in microsomes and 37.6% in the mitochondria) (Table 2). The supernatant fraction showed little activity (18.8%). Experiments on fatty acid specificity indicated that the incorporation of oleate acid was equal to that of palmitate

acid and that the incorporation of each of these was greater than that of linoleate acid (Table 3).

DISCUSSION

This study demonstrates for the first time the presence of cholesteryl esterifying activity in the gallbladder epithelium. The properties of the enzyme in terms of optimal pH and cofactor requirements are similar to those for cholesteryl ester synthetase in the liver (8,12). The absolute requirement for CoA and ATP suggests that the mechanism of the reaction operates through the formation of fatty acyl-CoA intermediates (8,12). The enzyme activity in gallbladder is distributed among mitochondrial and microsomal fractions similar to that seen in rat liver enzyme (8,13). The presence of the cholesteryl esterifying enzyme in the gallbladder and the observation of Neiderhiser et al. (4) that gallbladder can absorb cholesterol from the bile suggest a possible significance of this enzyme in normal animals and in particular under conditions when bile is supersaturated with cholesterol. This is emphasized by recent studies (1-4) showing that gallbladder is more than just an inactive tissue for biliary storage.

ACKNOWLEDGMENT

The authors are indebted to Dr. Bruce A. Kottke for his continued interest and suggestions, to Mr. William S. Harris for furnishing guinea pigs, and to Mr. James W. Penner and Miss Laurie K. Vongroven for assistance during the study. The study was supported in part by grant HL-14196 from the National Heart and Lung Institute.

REFERENCES

1. Diamond, J. M. (1968) in Handbook of Physiology. Section 6: Alimentary Canal, Code, C. F., and Heidel, W. (eds.) vol. 5, pp. 2451-2482, American Physiological Society, Washington, D.C.
2. Neiderhiser, D. H., Pineda, F. M., Hejduk, L. J., and Roth, H. P. (1971) J. Lab. Clin. Med, 77, 985-992.
3. Neiderhiser, D. H., Morningstar, W. A., and Roth, H. P. (1973) J. Lab. Clin. Med, 82, 891-897.
4. Neiderhiser, D. H., Harmon, C. K., and Roth, H. P. (1976) J. Lipid Res, 17, 117-124.

5. Small, D. M. (1967) Gastroenterology, 52, 607-610.
6. Goodman, D. S. (1965) Physiol. Rev, 45, 747-839.
7. Subbiah, M. T. R., and Dicke, B. A. (1976) Atherosclerosis, 24, 575-580.
8. Goodman, D. S., Deykin, D., and Shiratori, T. (1964) J. Biol. Chem, 239, 1335-1345.
9. Subbiah, M. T. R., and Dicke, B. A. (in press) Atherosclerosis.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem, 193, 265-275.
11. Subbiah, M. T. R., Unni, K. K., Kottke, B. A., Carlo, I. A., and Dinh, D. M. (1976) Exp. Mol. Pathol, 24, 287-301.
12. Mukherjee, S., Kunitake, G., and Alfin-Slater, R. B. (1958) J. Biol. Chem, 230, 91-96.
13. Deykin, D., and Goodman, D. S. (1962) Biochem. Biophys. Res. Commun, 8, 411-415.