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CONTRASTING EFFECTS OF VARIOUS SULFHYDRYL REAGENTS ON THE ACTIVITY OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE

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SUMMARY

Lecithin:cholesterol acyltransferase, E.C. 2.3.1.43, can be either stimulated or inhibited by reducing reagents containing free sulfhydryl groups. Mercaptoethanol and dithiothreitol stimulate enzyme activity while cysteine and reduced glutathione inhibit activity. The oxidizing disulfide reagent 2-pyridine disulfide has minimal effects on enzyme activity by itself but suppresses the stimulatory effect of mercaptoethanol.

INTRODUCTION

Lecithin:cholesterol acyltransferase (LCAT)*, is the plasma enzyme apparently responsible for the synthesis of most plasma cholesterol esters from plasma unesterified cholesterol and lecithin (1). Each molecule contains about 6 cysteine residues (2), and is sensitive to a variety of sulfhydryl reactive compounds. Mercaptoethanol (ME) stimulates, (3), 5,5'-dithiobis-(2-nitrobenzoic acid) and p-hydroxymercuribenzoate reversibly inhibit (4,5) and N-ethylmaleimide (NEM) irreversibly inhibits (5) LCAT activity. An interpretation of these previous results is that LCAT contains a free sulfhydryl group which when oxidized leads to inactivation of the enzyme.

In the studies reported below effects of various other sulfhydryl reagents on LCAT activity were examined. The stimulatory effect of ME was ver-

*Abbreviations used: lecithin:cholesterol acyltransferase, E.C. 2.3.1.43, LCAT; mercaptoethanol, ME; dithiothreitol, DTT; 2-pyridine disulfide, PDS; N-ethylmaleimide, NEM; cysteine, CysSH; reduced glutathione, GSH.

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ified. In addition, it was shown that the reducing agent dithiothreitol (DTT) stimulates LCAT activity. However two other reducing agents, cysteine (CysSH) and reduced glutathione (GSH), inhibit LCAT activity. Furthermore, the mild sulfhydryl oxidizer 2-pyridine disulfide (PDS), does not affect LCAT activity by itself, but suppresses the stimulatory effect caused by ME to an extent greater than its effect on the free sulfhydryl-disulfide equilibrium. These results show that the role of sulfhydryl groups in LCAT activity is considerably more complex than previously described.

MATERIALS AND METHODS

LCAT was partially purified by centrifugation (6). Normal human plasma was adjusted to a density of 1.25 g/ml by addition of KBr and centrifuged for 48 h at 50,000 rpm in a Ti-60 rotor (Beckman, Palo Alto, CA). The lipoprotein fraction was removed and recentrifuged under the same conditions at a density of 1.21 g/ml. The infranatant fraction containing LCAT free of most lipoproteins and albumin was frozen at -70°C and stored until used. This LCAT preparation was approximately 250 times purified with respect to plasma activity, and had a protein concentration of about 1 mg/ml. Immediately before each experiment an aliquot was thawed and dialyzed against buffer containing 10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, and 0.02% NaN_3.

LCAT activity was measured using a rapid assay technique previously described (7,8). Briefly, liposomes of lecithin and cholesterol, 2:1 lecithin: cholesterol mole ratio, containing tracer amounts (1 μCi/μmole) of uniformly labelled [^{14}C]lecithin (New England Nuclear, Boston, MA) were prepared by the method of Batzri and Korn (9) in buffer containing 10 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.02% NaN_3 and sulfhydryl reagents as described in the text. 100 μl of the vesicle preparation containing 25 nmole lecithin were added to 100 μl of LCAT and incubated at 37°C for 30 min. The reaction was stopped by addition of 2.0 ml of Dole's mixture (10), isopropanol:heptane: 1N H_2SO_4, 400:100:10. After addition of 1.2 ml heptane and 1.0 ml water two phases were obtained by centrifugation. 1.0 ml of the upper heptane phase was added to 2.0 ml heptane and mixed with about 100 mg of silicic acid which adsorbs extracted lecithin and reduces the blank (11). The heptane, containing neutral lipid, was then counted in 10 ml Liquifluor (New England Nuclear, Boston, MA). Results were calculated on the basis of the specific activity of the 2'-acyl group of lecithin as determined by hydrolysis with snake venom, Crotalus adamanteus (Sigma, St.Louis, MO) (12). Since total production of neutral lipids was determined, both the transferase and phospholipase (13,14) activities of LCAT were included in this measurement. However, under the conditions used, phospholipase activity was less than 10% of the transferase activity (8).

Sulfhydryl reagents were obtained from Sigma (St.Louis, MO). Concentrations of free sulfhydryl were standardized against mercaptoethanol using PDS (15).

RESULTS

At a concentration of 10 mM various sulfhydryl reagents had different effects on the activity of LCAT. Table I shows that ME stimulated LCAT activ-

TABLE I

Effect of Various Sulfhydryl Reagents on Activity of LCAT

Sulfhydryl Reagent	LCAT (pmol/h/ml)
none	1560 ± 150
10 mM mercaptoethanol	4170 ± 190
10 mM cysteine	0 ± 40
10 mM 2-pyridine disulfide	1300 ± 30
10 mM dithiothreitol	2270 ± 130

LCAT was measured as described in the text using liposomes made in the presence of the indicated sulfhydryl reagent. Concentrations are final concentrations in the reaction mixture. Activity is expressed per ml of semipurified LCAT, approximately 1 mg of protein. Data are means ± 1 standard deviation.

ity about 3-fold, CysSH inhibited LCAT activity, PDS had a mild inhibitory effect, and DTT stimulated LCAT activity about 1.5 fold. The inhibitory effect of CysSH was unexpected because it had been assumed that ME stimulated LCAT activity by reducing an oxidized form of the enzyme; and CysSH has a similar reducing ability.

To look more closely at the inhibitory effect of CysSH and to consider the possibility that another naturally occuring sulfhydryl reagent, GSH, might also affect LCAT activity, the effect of these two reagents and ME on LCAT activity was determined as a function of their concentrations. Figure 1 shows that, while ME stimulated LCAT activity, both CysSH and GSH inhibited LCAT activity. GSH appeared to be more effective at low concentrations than CysSH in inhibiting LCAT.

The slight inhibitory effect of PDS suggested that it affected LCAT activity by reversibly oxidizing LCAT. To investigate this further, the effect of mixtures of PDS and ME on LCAT activity was determined. Table II shows that

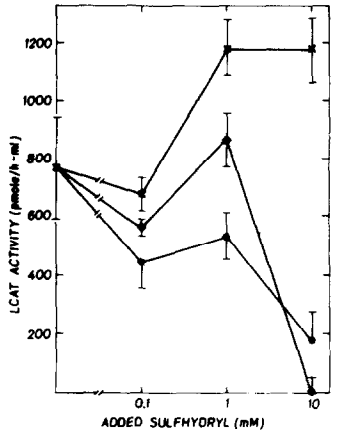

Figure 1. Effects of different concentrations of mercaptoethanol, X——X, cysteine, ◆——◆ , and glutathione, ●——● , on LCAT activity. Liposomes were prepared using buffers containing 2X the indicated concentration of sulfhydryl reagent. Activity of semipurified LCAT was determined using these liposomes as described in materials and methods. 1 ml of this semipurified LCAT contained about 1 mg of protein. Data are means ± 1 standard deviation.

ME stimulated LCAT activity and PDS inhibited it slightly as described above. When mixtures of 1 mM ME and 2 mM PDS were tested for their effect on LCAT it was seen that the PDS completely eliminated the stimulatory effect of ME.

TABLE II

Effect of Mercaptoethanol on Inhibition of LCAT by Cysteine

Sulfhydryl Reagent	LCAT (pmol/h/ml)
none	640 ± 50
10 mM cysteine	40 ± 3
10 mM cysteine + 1 mM mercaptoethanol	40 ± 3
10 mM cysteine + 10 mM mercaptoethanol	120 ± 3

LCAT was measured as described in the text with various concentrations of cysteine and mercaptoethanol included in the reaction mixture. Activity is expressed per ml of semipurified LCAT, approximately 1 mg of protein. Data are means ± 1 standard deviation.

This was true whether ME was added to an assay mixture containing PDS or whether PDS was added to an assay mixture containing ME. At these concentrations it appeared that PDS eliminated the effect of ME by changing the free sulfhydryl-disulfide equilibrium. However, when the effect of 2 mM PDS on the stimulatory effect of 10 mM ME was considered it was seen that the stimulatory effect of ME was reduced more than expected. On the other hand, increasing the amount of PDS to 10 mM did not cause a further reduction in the activity of the enzyme.

Because ME was able to eliminate the inhibitory effect of PDS and is known to reverse the effect of other disulfide inhibitors, the effect of ME on the inhibition caused by CysSH was investigated. Table III shows the results of assaying LCAT in the presence of CysSH and different amount of ME. These results show that ME was not able to reverse the inhibition of LCAT caused by CysSH, although a slight increase could be seen when 10 mM ME was added to 10 mM CysSH.

DISCUSSION

From this study and previous results, it appears that five different effects of sulfhydryl reagents on LCAT activity can be listed. Reagents containing free sulfhydryl groups can stimulate or inhibit LCAT activity, reagents containing disulfide groups can reversibly inhibit LCAT activity or inhibit the stimulatory effect of reagents containing free sulfhydryl groups, or reagents such as NEM can irreversibly inhibit LCAT activity. The mechanisms responsible for these various effects are unclear. The contrasting effects of different reducing agents however, suggest that both free sulfhydryl and disulfide linkages in LCAT are necessary for LCAT activity.

One interpretation of effects of mixtures of PDS and ME and CysSH and ME on LCAT activity is that certain mixed disulfides of LCAT are inactive while others are active. This would explain the contrasting effects of CysSH and ME since a mixed disulfide with CysSH (or GSH) would contain extra charged

TABLE III

Effect of Mixtures of 2-Pyridine Disulfide and Mercaptoethanol on LCAT activity

Sulfhydryl Reagent	Relative LCAT activity
none	100%
1 mM mercaptoethanol	210
1 mM mercaptoethanol + 2 mM 2-pyridine disulfide*	130
2 mM 2-pyridine disulfide + 1 mM mercaptoethanol*	120
10 mM mercaptoethanol	330
10 mM mercaptoethanol + 2 mM 2-pyridine disulfide	230
10 mM mercaptoethanol + 10 mM 2-pyridine disulfide	210
2 mM 2-pyridine disulfide	110
10 mM 2-pyridine disulfide	80

LCAT was measured as described in the text using liposomes made in the presence of the indicated sulfhydryl reagents except for those indicated* where liposomes were made in the presence of the first reagent and the second was added after the LCAT. Concentrations are final concentrations in the reaction mixture. 100% activity equaled 1520 ± 170 pmol/h/ml.

groups whereas a mixed disulfide with ME would not. Further studies on intramolecular disulfide bonds in active and inactive pure LCAT would perhaps answer these questions.

The physiological significance of these observations is difficult to determine. Both reduced cysteine and oxidized cystine (16) and reduced and oxidized glutathione (17) are present in plasma. Further, it is known that a particular free sulfhydryl-disulfide equilibrium is maintained in plasma (18)

and that this equilibrium changes on exposure of plasma to air (19). However, its importance is unknown.

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