A METHOD FOR STUDYING THE INTERACTION BETWEEN LECITHIN:CHOLESTEROL ACYLTRANSFERASE AND HIGH DENSITY LIPOPROTEINS

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High density lipoproteins (HDL) are preferred substrates of the lecithin:cholesterol acyltransferase (LCAT) of blood plasma (Glomset, 1968). Although the basis of this preferential action of LCAT has not been clarified, presumably it depends on the formation of specific enzyme-substrate complexes. In this communication we describe a method for studying the formation of LCAT-HDL complexes that should prove useful not only in investigations of the mechanism of the acyltransferase reaction and the structure of HDL, but also in attempts to purify the enzyme. The method involves the formation of HDL-agarose, and the subsequent utilization of this insoluble, but apparently fully hydrated, product in studies of the interaction between HDL and LCAT. The experiments described below specifically concern the disruption of LCAT-HDL complexes by sodium taurocholate.

MATERIALS AND METHODS

LCAT was partially purified from human plasma and assayed as described earlier (Glomset and Wright, 1964). HDL were prepared by ultracentrifuging human plasma essentially according to Havel, Eder, and Bragdon (1955) except that solid KBr was used to adjust the density of the plasma and that HDL were

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floated at a density of 1.25 g/ml. The HDL were washed three times by refloatation and dialysed against 0.14 N NaCl. Finally, the concentration of the HDL solution was adjusted so that the absorbance at 280 mμ was 8.5 , and the HDL were coupled to Sepharose 2B (Pharmacia Fine Chemicals, Piscataway, New Market, New Jersey) by the method of Porath et al. for coupling proteins to cyanogen bromide-activated agarose. Approximately 0.5 ml HDL solution were used per g wet weight of Sepharose.

Lipids were analyzed as described earlier (Glomset et al., 1966). The sodium taurocholate was a gift from Dr. David Saunders. Taurocholate analyses were performed by Dr. H. P. Porter, essentially by the method of Iwata and Yamasaki (1964).

RESULTS AND DISCUSSION

Table 1 shows the yields of HDL-agarose obtained in three separate experiments. In preliminary experiments we incubated the HDL-agarose in pH 7.4 Tris-HCL buffer (0.01 M TrisHCL - 0.14 N NaCl - 0.001 M EDTA) in the presence and absence of LCAT. No changes in HDL-agarose lipid occurred during a 24 hour incubation at 37°C. in the absence of the enzyme, indicating that the HDL-agarose was relatively stable. However, in the presence of the enzyme

TABLE 1

Experiment	μMoles lipid*		Yield (%)	
	Cholesterol	Lecithin	Cholesterol	Lecithin
1	1.4	1.0	48	49
2	1.3	0.7	50	45
3	0.7	0.4	46	40

* per gram wet weight HDL-agarose

esterification of the cholesterol of the HDL-agarose occurred, indicating that reactive enzyme-substrate complexes had been formed.

Figs. 1, 2, and 3 directly demonstrate the formation of LCAT-HDL complexes and their disruption by sodium taurocholate. In the experiment shown in fig. 1 equal amounts of partially purified LCAT were applied to a column of HDL-agarose and two control columns that contained untreated agarose and cyanogen bromide-activated agarose, respectively. The fact that the effluent

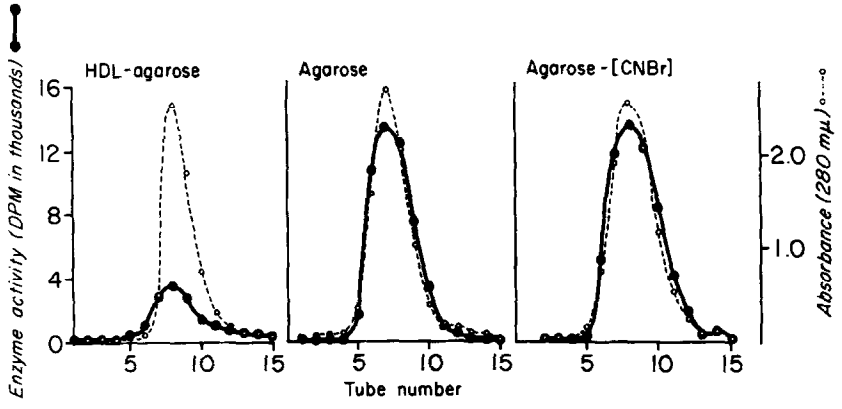

Fig. 1

Equal amounts of partially purified LCAT (approximately equivalent to the activity in 8 mls fresh human plasma) were applied to columns containing 8 g HDL-agarose, untreated agarose, and cyanogen bromide-treated agarose, respectively. The columns were washed with Tris HCl-NaCl-EDTA, pH 7.4 at 4°C., and the absorbance at 280 mμ and the LCAT activity of the column effluents were measured.

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from the HDL-agarose column contained approximately the same amount of protein as the effluent from the control columns, but much less LCAT activity, is consistent with specific binding of LCAT and HDL. The occurrence of specific binding is further indicated by the experiment shown in fig. 2, in which the same three columns shown in fig. 1 were eluted with 5 mM sodium taurocholate. This elutrient was chosen because of its potent inhibitory effect on the acyltransferase reaction (Sperry and Stoyanoff, 1937), and because preliminary experiments had shown it to be much more effective in disrupting LCAT-HDL complexes than other inhibitors of the acyltransferase reaction such as para-

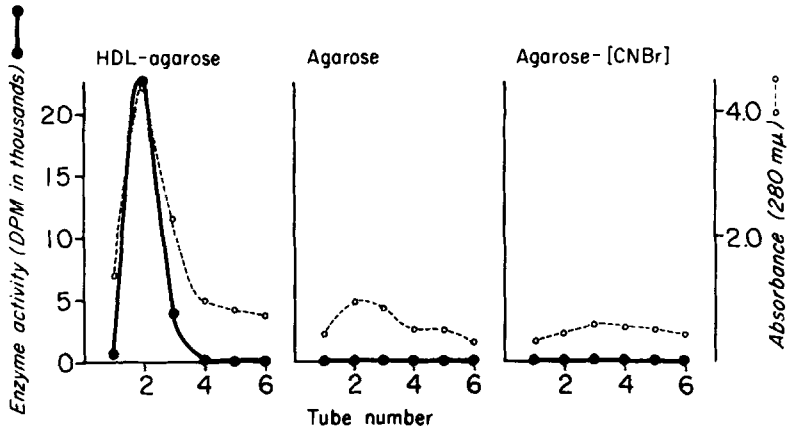

Fig. 2

The columns from the experiment shown in fig. 1 were eluted at room temperature with 5mM sodium taurocholate, each effluent subfraction was dialyzed against Tris buffer, and the absorbance and LCAT activity were measured.

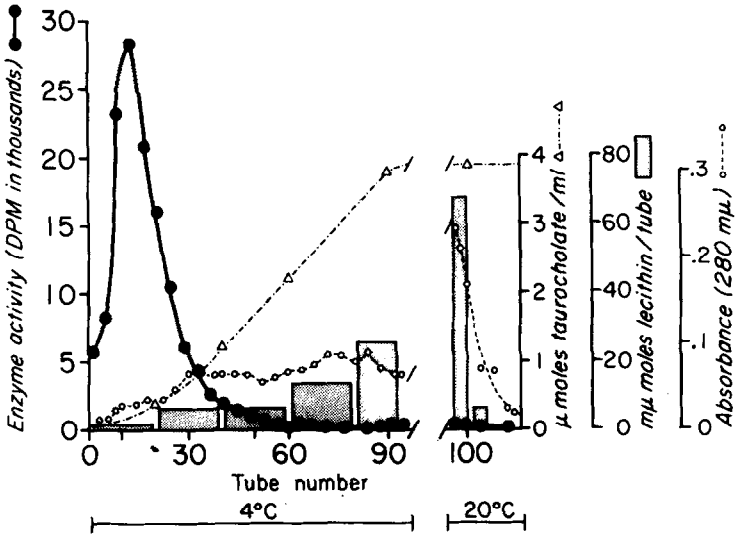

Fig. 3

Partially purified LCAT was applied to a column containing HDL-agarose and the column was washed with Tris buffer as in the experiment shown in fig. 1. Then the column was eluted with an approximately linear gradient of sodium taurocholate at 4°C and subsequently at room temperature.

chloromercuriphenylsulfonate, 3 M KCl, or 0.1 M $CaCl_2$. In addition to disrupting the LCAT-HDL complex, the sodium taurocholate caused the release of lipid from the HDL-agarose column. However, the experiment shown in fig. 3 shows that these two effects can be dissociated. In this experiment partially purified LCAT was applied to a column of HDL-agarose and the latter was washed with Tris buffer. Then an approximately linear gradient of sodium taurocholate was applied at 4°C. As can be seen the enzyme was released at lower concentrations of taurocholate than most of the u.v. absorbing material or lecithin. This experiment suggests that adsorption of LCAT on HDL-agarose followed by elution with approximately 0.5 mM taurocholate might be an effective means of purifying the enzyme.

The nature of the linkage between LCAT and HDL-agarose remains to be established. However, HDL apoprotein may be involved, since preliminary experiments have shown that LCAT binds to HDL apoprotein-agarose. Experiments are in progress to determine the role of HDL lecithin and unesterified cholesterol in the binding of LCAT to its lipoprotein substrate.

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