LCAT is a ~66 kDa-glycoprotein secreted by the liver and catalyzes the formation of most plasma cholesterol esters. The secondary LCAT deficiency of liver disease generates abnormal lipoprotein particles which have several adverse effects on cellular functions [1]. Although this reduction in LCAT activity may largely reflect low LCAT mass [2], other serum glycoproteins have a decreased sialic acid content and increase in glycan branching [3]. Conceivably, hepatic dysfunction may also affect LCAT glycosylation and enzymic activity. However, a study of LCAT in individuals is complicated by its purification which usually requires multi-step chromatography and large volumes of plasma [4]. In an alternative approach, we demonstrate herein the feasibility of isolating LCAT from small volumes of cirrhotic plasma and of carrying out subsequent microheterogeneity studies.

Four patients with hepatic cirrhosis (1, alcoholic; 2, primary biliary; 1 cryptogenic) were studied using freshly-prepared EDTA-plasma. Total IgG was isolated from rabbit anti-human LCAT antisera [4] and cross-linked to CNBr-activated Sepharose (5 mg/ml). Plasma (1 ml) was equilibrated with anti-LCAT-Sepharose (2 ml) for 1 h at 4°C in an end-over-end mixer, transferred to a column and unbound material eluted with phosphate-buffered saline. Bound LCAT was eluted with 0.5 M NaCl, concentrated to 200 μl and analyzed by SDS-PAGE with silver staining. In some cases, the eluate was applied to a con A-Sepharose column, eluting any bound material with 0.2 M α-methylmannoside. LCAT activity was measured by the proteoliposome method [5].

LCAT protein (confirmed by Western blotting [4], data not shown) was readily detected in two healthy subjects (Fig. 1A, lanes 2 & 3) following immunoaffinity chromatography of their plasma. LCAT was also detectable in the three cirrhotic plasmas (lanes 3-5) but two of the patients had substantially reduced levels (lanes 4 & 6). One patient with primary biliary cirrhosis was studied before and 7 weeks after orthotopic liver transplantation; her plasma LCAT activity rose from 20 to 88 nmol/ml/h and a clear increase in LCAT mass was seen (Fig. 1B; lane 3, post-transplant vs. lane 2, pre-transplant).

![Image 1](image1.png)

**Fig. 1.** Characterization of immunoaffinity-isolated LCAT by SDS-PAGE and silver staining. Sample details are given in the text while molecular mass markers (kDa) are shown in lanes 1; the arrows indicate LCAT. Note that in B, the bands immediately below LCAT were reagent contaminants as they also occurred in the markers.

Imunoaffinity-isolated LCAT from a patient with cryptogenic cirrhosis (Fig. 2, lane 1) was also applied to a con A-Sepharose column but failed to bind to this lectin (lane 2, the 0.2 M α-methylmannoside eluate). By contrast, a small amount of LCAT from a patient with alcoholic cirrhosis did appear to interact with con A (lane 4), although most LCAT was unbound (lane 3).

![Image 2](image2.png)

**Fig. 2.** Interaction of immunoaffinity-isolated plasma LCAT with con A-Sepharose. See text for details.

This study describes the first use of an anti-LCAT immunoaffinity column to isolate LCAT from cirrhotic plasma. Some contaminants were still present, perhaps because plasma LCAT is intimately associated with HDL proteins or because some antibodies may be directed against N-glycan epitopes common to other serum glycoproteins. Patient and normal LCAT had an indistinguishable electrophoretic mobility by SDS-PAGE (Fig. 1A), implying a comparable molecular mass and total carbohydrate content. Similarly, no mobility shift in plasma LCAT was noted pre- and post-transplantation, although the concentration of LCAT clearly increased.

The four N-linked oligosaccharide chains (Asn20, Asn84, Asn272, and Asn290) on normal plasma LCAT have recently been characterized as sialylated triantennary and/or biantennary complex structures by HPLC/electrospray mass spectrometry [6]. No high-mannose chains were detected, refuting an earlier report using endoglycosidase H [7], possibly because this enzyme was contaminated with endo F [6]. The failure of normal LCAT (not shown) and LCAT from one cirrhotic patient (Fig. 2) to interact with con A supports this conclusion; this lectin preferentially binds high-mannose, rather than complex-type, carbohydrate chains. However, LCAT from another patient did appear to interact with con A, implying that some LCAT molecules contain immature oligomannose chains. Whether this preliminary evidence of LCAT microheterogeneity in hepatic cirrhosis can be confirmed by direct analysis or by more sensitive techniques must await further studies.

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