DE-ESTERIFICATION OF CHOLESTERYL ESTERS IN HUMAN PLASMA
α-LIPOPROTEIN (HDL) BY PREPARATIONS OF STAPHYLOCOCCAL ALPHA TOXIN

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SUMMARY

Chemical extraction and analysis of reaction mixtures of preparations of
staphylococcal alpha toxin and human plasma α-lipoprotein (HDL) indicated that
the cholesteryl esters which form an integral part of the lipoprotein molecules
were de-esterified by the toxin preparations with no apparent destruction of the
cholesterol moiety.

This report is the first of cholesterol esterase produced by staphylococcus.
This activity is closely associated with alpha toxin and may be a manifestation
of alpha toxin itself. In addition it is shown that soluble plasma lipoproteins
are useful substrates for biological reactions involving lipids.

Certain bacteria produce extracellular toxic proteins which are cytolytic,

causing damage to the structural and functional integrity of membrane systems of

eucaryotic and in some cases, procaryotic cells (1). The alpha toxin of Staphylo-

coccus aureus is one such agent. It is a heat labile protein whose monomeric form

has a sedimentation rate of 3S, which aggregates easily to a soluble, less active

12S form and eventually to an insoluble, inactive form (2). Although the second-

ary effects resulting from the action of alpha toxin on a variety of natural and

artificial membrane systems have been described (3), the primary site and mechanism

of membrane damage has not been reported.

During investigations on the effect of membrane damaging agents on soluble

lipoproteins, I have obtained data indicating that preparations of staphylococcal

alpha toxin had a specific action of de-esterifying the cholesteryl esters which

are an integral part of lipoprotein molecules (4). The cholesteryl moiety itself

does not seem to be altered by the toxin.

Materials and Methods

Alpha toxin, produced by the Wood 46 strain of Staphylococcus aureus was

purified by ammonium sulfate precipitation and by successive column chromatography
over G-50 fine and then G-100 super fine Sephadex gels (5,6). Analytical ultra-
centrifuge diagrams of all preparations showed either a single 3S peak character-
istic of monomeric alpha toxin or the 3S peak accompanied by a 12S peak character-
istic of aggregated alpha toxin. Preparations used in this study were free of
proteolytic activity by the Azocol method (Calbiochem, La Jolla, California, Docu-
ment No. 3505). Hemolytic activity was assayed using rabbit red blood cells (5).

Human plasma α-lipoprotein (HDL) was isolated and purified by differential
flotation and equilibrium density gradient purification (7,8). It was quantitated
using an $E_{1cm,280 \, nm}^{1\%}$ of 5.5 based on dry weight measurements. Polyacrylamide gel
electrophoresis of protein preparations was performed in 7-1/2% gels and at pH 8.9
(9) using a Buchler Polyanalyst apparatus. Lipids were extracted from lipoproteins
by chloroform-methanol (10). Separation of extracted esterified and unesterified
cholesterol was done by silicic acid chromatography (11) and the resultant fractions
were analyzed spectrophotometrically (12).

Experimental Procedure

The experimental reaction mixtures contained a known amount of α-lipoprotein
(HDL) (ca 10 mg/ml) and amounts of alpha toxin preparation containing from 3,000 to
15,000, 50% hemolytic units per mg. lipoprotein.

The mixtures were incubated at 37°C in phosphate buffer at pH 7.0 (5) for 2
hours. Controls contained the same concentration of α-lipoprotein (HDL) without
added alpha toxin. At the end of the incubation period samples of the reaction mix-
ture and of the control mixture were examined by electrophoresis in polyacrylamide
gels, by analytical ultracentrifugation and by extraction and chemical analysis of
lipids.

Results

The results of the chemical analyses showed that α-lipoprotein (HDL) treated
with the alpha toxin preparations contained less esterified cholesterol and propor-
tionately more unesterified cholesterol as shown in Table 1. The decrease in
esterified cholesterol became more pronounced as the ratio of alpha toxin to α-

De-esterification of Cholesteryl Esters in Human Plasma

α-Lipoprotein by Staphylococcal Alpha Toxin[a]

| Units[b] of alpha toxin | Cholesteryl Moiety[c] | | | | Molar Ratio |
| mg. lipoprotein | µg/mg lipoprotein | | | Percent | Esterified to |
	Unesterified	Esterified	Total	Recovered	Total Cholesterol
Control					
0 (i)	32	88	120	-	0.73
0 (ii)	24	73	97	-	0.75
Test					
3×10^3 (i)	46	80	126	105	0.63
8×10^3 (ii)	67	34	101	104	0.34
15×10^3 (i)	89	7	96	80	0.07

[a] Each experiment used a different preparation of alpha toxin. Two preparations of α-lipoprotein were used, designated (i) or (ii). Each experiment was incubated at 37°C for 2 hours, pH 7. To convert the esterified values in column 3 into weight of the ester, these figures should be multiplied by 1.72 (4).

[b] Rabbit Red Blood Cell hemolytic units (5).

[c] The cumulative reproducibility of methods of extraction, separation and chemical analysis of cholesterol is, in my laboratory better than 5% over an extended period of time.

lipoprotein (HDL) was increased. Alpha toxin preparations held at 60° for 2 minutes or at room temperature for 12 hours had lost both their lytic activity on rabbit red blood cells and their de-esterifying activity on HDL. The data show that in experiments where the ratio of alpha toxin to lipoprotein was lower, i.e., 3×10^3 and 8×10^3 H.U./mg, the weight recoveries of the cholesteryl moiety (esterified + unesterified) in the test as compared with the control mixtures were essentially the same. This observation is evidence that the decrease in the amount of esterified cholesterol in the α-lipoprotein (HDL) which had been treated with alpha toxin preparations was not the result of some toxin-induced loss of the assay-reactive portion of the cholesteryl moiety or of the loss of intact cholesteryl ester. The lower recovery (80%) in the experiment containing 15×10^3 H.U. alpha toxin/mg
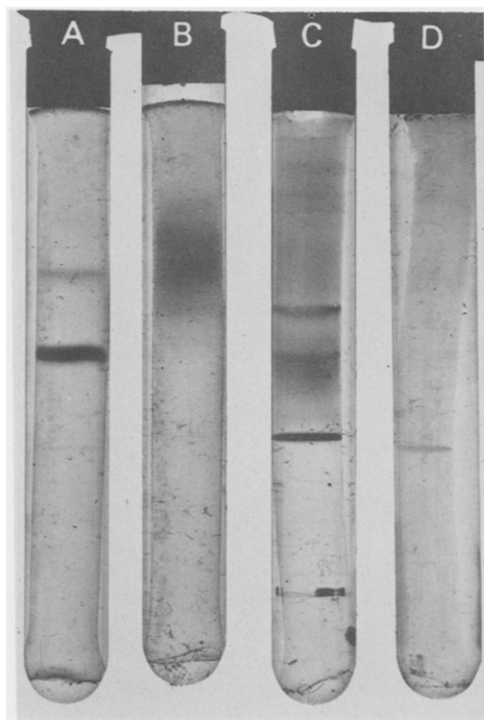
Figure 1. Polyacrylamide gel electrophoresis in 7.5% gels at pH 8.9.
a. bovine serum albumin, included as a reference. b. α-
lipoprotein (HDL) MW ca 300,000. The broad band in this
gel is due to both the high molecular weight and to the
broad density and size distribution of pure α-lipoprotein
(HDL)(4). c. α-lipoprotein (HDL) - alpha toxin mixture.
The α-lipoprotein was incubated for 2 hours at 37°, with
8000 H.U. alpha toxin per mg. lipoprotein. d. alpha
toxin, same concentration as in c.
Note: The black spots at the bottom of gel c are artifacts.

lipoprotein may have been due to sampling difficulties due to denaturation and

fragmentation of the lipoprotein as seen visually and in the analytical ultracentri-

fuge. Additional data indicate that as the reaction between α-lipoprotein (HDL)

and alpha toxin preparations proceed the lipoprotein molecule begins to lose its

integrity, releasing some smaller fragments and leaving a residual molecule(s) of

lower sedimentation rate. The electrophoretic mobility of these fragments after

polyacrylamide gel electrophoresis is increased compared to that of the unreacted

lipoprotein control (Figure 1).

Discussion

The findings presented in this report indicate that our staphylococcal alpha toxin preparations de-esterify cholesteryl esters present as a part of plasma α-lipoprotein (HDL). A result of this reaction is a gradual fragmentation of the lipoprotein molecule. The fact that the esterase activity has not been described previously may be ascribed to the emphasis placed on studying the various secondary effects of alpha toxin because these effects may be important in naturally affected membrane systems. Furthermore, studies using cholesteryl esters alone are difficult due to the insolubility of these molecules when present in aqueous systems.

Observations relevant to the role of cholesteryl esters in the stability of cell membranes have indicated that the red blood cell membranes of individuals suffering from lecithin-cholesterol-acyl-transferase (LCAT) deficiency have a lowered cholesteryl ester content. When serum containing normal LCAT activity is added to these abnormal cells the proportion of cholesteryl ester is increased to normal. The red cells of LCAT deficient blood are also abnormal in shape and have an increased fragility (13). Other studies have shown that red cells become more fragile as the content of cholesteryl ester decreases (14).

The task of proving that a single molecular species carries more than one biological activity is arduous. Detailed experiments are in progress in my laboratory designed either to prove or to disprove the identity of staphylococcal alpha toxin and the cholesteryl esterase described here. In either case, it is evident that Staphylococcus aureus does produce a cholesterol esterase and that soluble lipoproteins provide a useful model system for the study of biological reactions involving lipids.

Acknowledgments

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