Opacity factor from group A streptococci is an apoproteinase

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1. SUMMARY

Opacity factor (OF) is an enzyme, elaborated by certain serotypes of group A streptococci, which produces opalescence in mammalian sera. OF has been designated a lipoproteinase. Lipoproteins are complex structures and many enzymes are involved in their catalysis. We therefore set out to establish which of the many enzymes OF could be. Results showed that OF rendered high density lipoprotein (HDL) insoluble, accounting for the opalescence in serum, and altered its electrophoretic mobility. Electron microscopy revealed that OF caused an aggregation of HDL and an alteration in molecule shape. OF specifically split apoprotein AI of HDL into two fragments demonstrable by SDS-PAGE. We therefore designate OF as an apoproteinase.

2. INTRODUCTION

Although opacity factor (OF) typing by an opacity-inhibition test is used as a supplementary system for typing of OF-producing group A streptococci, the exact enzymic nature of the serum opacity reaction has not been established. Suggested enzymic actions for OF have been serum protein denaturation [1], lipoproteinase activity [2], and cholesterol esterification [3]. Although the term opacity factor generally has been adopted, a number of authors have referred to OF as a lipoproteinase [4–6]. The use of the name lipoproteinase seemed inappropriate because of the complex nature of human serum and the involvement of a large number of enzymes in the catalysis of lipoproteins. We therefore set out to establish the enzymic nature of OF.

3. MATERIALS AND METHODS

3.1 OF producing strain

Streptococcus pyogenes (R68/3116, T12, M22) obtained from the PHLS, Colindale, U.K. The organism was grown as described previously [7].

3.2 OF preparation

OF from cell-free supernatant was purified by ammonium sulphate precipitation, DE-52 anion-exchange cellulose, and sephacryl S-400 column chromatography [8].
3.3 High density lipoprotein (HDL)

Purified by ultracentrifugation at \( d = 1.21 \); containing 5 mg \( \cdot \) ml\(^{-1} \) apoprotein AI (Apo AI); was a gift from Dr. P. George of Clinical Biochemistry, Christchurch Hospital, Christchurch, New Zealand.

3.4 OF activity on various substrates

OF activity was determined using both the purified enzyme and by the culturing of the OF producing strain directly on the substrate. Opalescence in serum and in purified HDL was assayed using serum agar [9] and optical density methods [10]; proteinase activity was measured on casein-milk agar (0.2% : 2%) and bovine-albumin-milk agar (0.2% : 2%) [11]. Results were compared against the activity of trypsin and proteinase K. Lipase activity was assayed on Sierra's medium [12] with two wild-type strains of *Staphylococcus aureus* as control producers. Phospholipase activity was measured on McClung-Toabe egg yolk agar [13] with a strain of *Bacillus cereus* as control producer. Cholesteryl esterhydrolase activity was assayed by measuring free cholesterol and fatty acids following treatment of cholesterol oleate [14].

3.5 Products of enzyme activity

Both human and horse sera were treated with OF. Free and esterified cholesterol and fatty acids were analysed by thin-layer chromatography using TLC-plastic sheet silica gel 60 (Prod No 5506, Appl. Sci. Lab., U.S.A.) to indicate lecithin-cholesterol acyltransferase (LCAT). Fatty acids were extracted by hexane-ether (1 : 1 v/v), methylated with BF 3-methanol, and analysed by Pye-Unicam gas chromatography [15] to detect catalytic action on lipids by phospholipases A1, A2, and B, lipoprotein lipase and cholesteryl esterhydrolase. Triglycerides were extracted [16] and free glycerol determined using an assay kit (Prod No 6097, Abbott Lab, U.S.A.) to detect hydrolysis of chylomicrons indicating lipoproteins lipase.

3.6 Electrophoresis

OF-treated and untreated serum samples were electrophoresed (0.75% agarose) to observe changes in lipoproteins and proteins [17]. SDS-PAGE (method of Laemmli [18]) of OF-treated and untreated HDL was used to examine changes in apoprotein.

3.7 Electron microscopy

OF-treated and untreated Purified HDL were negatively stained by 2% sodium phosphotungstate (pH 7.4) and examined using a JEM-100CX (Jeol Ltd, Japan) electron microscope.

4. RESULTS AND DISCUSSION

With the exception of serum, OF had no measurable activity on the substrates tested. Failure to detect appropriate end products of enzyme activity indicated that OF was not a lipase, phospholipase, lecithin-cholesterol acyltransferase, cholesteryl esterhydrolase, not lipoprotein lipase. Three significant changes were observed following OF treatment of serum and of purified HDL. These were: (i) The appearance of opalescence, (ii) changes in the electrophoretic mobility of HDL.

![Fig. 1. Electrophoretic separation of serum lipoproteins on agarose. A and I, OF-treated HDL; B and J, untreated HDL; C and E, OF-treated human sera; D and F, untreated human sera; G, OF-treated horse serum; H, untreated horse serum.](image-url)
Fig. 2. SDS-PAGE of purified HDL-treated at 37°C with: OF (136 μg·ml⁻¹) A (4 h) and B (16 h), trypsin (200 μg·ml⁻¹) E (4 h) and F (16 h); C untreated HDL. D standard markers [(1) phosphorylase b, 94000; (2) bovine serum albumin, 67000; (3) ovalbumin, 43000; (4) carbonic anhydrase, 30000; (5) soybean trypsin inhibitor, 20000].

Fig. 1, (iii) the splitting of apo AI (the major protein component of HDL with molecular weight of 28000) into two fragments (Fig. 2). We believe these results indicate that OF nicks the apo AI of the HDL to expose the highly insoluble centre core, causing the observed changes in solubility and in electrophoretic mobility.

A shell model for the structure of HDL has been proposed by various workers [19–20]. In the model HDL, consists of a centre core with a relatively low-electron density containing the neutral lipids and a surrounding outer shell of high-electron density containing the protein and phospholipid components. In our electron microscopic studies OF-treated HDL appeared aggregated (Fig. 3), a result consistent with hydrophobic interaction of exposed centre cores of HDL particles. Although larger and flatter in shape than untreated particles, the HDL retained their spherical shape. It has been shown that in persons with LCAT deficiency HDLs are disc-shaped, explained by the absence of cholesteryl esters in the core of the HDL structure [21]. We found no disc-shaped particles, suggesting that cholesteryl ester had remained intact in the complex (Fig. 3).

OF enzyme activity clearly differed from that of other proteinases tested, in that these enzymes completely degraded apo AI (Fig. 2) and did not produce similar serum opacity. Our results demonstrated that OF specifically cleaved apo AI
Fig. 3. Electron micrograph of human serum HDL. A, OF-treated HDL; B, untreated HDL. Arrows indicate aggregated HDL. Scale indicates 100 nm.

(Fig. 2) and did not hydrolyse other serum proteins (results not shown). We therefore propose the name apoproteinase. Further studies on OF characterisation and the cleavage site on apoprotein AI are in progress.

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REFERENCES
