A PROTEIN COMPONENT OF SERUM HIGH DENSITY LIPOPROTEIN WITH CO-FACTOR ACTIVITY AGAINST PURIFIED LIPOPROTEIN LIPASE

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Received April 21, 1970

SUMMARY

The ability of human serum HDL_2† (d 1.063-1.125), its lipid-free derivative, apo HDL_2, and subfractions to activate the hydrolysis of triglycerides by lipoprotein lipase was investigated. The activity of apo HDL_2, significantly reduced with respect to HDL_2, was found almost completely in the low molecular weight fraction (Sephadex fraction V) making up about 5% of apo HDL_2. On the other hand, the principal protein present in apo HDL_2 (fraction III) was totally devoid of such activity. Experiments using lipid-enriched products from sonically irradiated HDL_2 or mixtures of apo HDL_2 and its lipids led to similar conclusions.

Lipoprotein lipase (LPL) (EC 3.1.1.3), either extracted from tissues or highly purified from post-heparin plasma, has been shown to hydrolyze artificial triglyceride emulsions only in the presence of a plasma lipoprotein co-factor (1-3). The high density lipoprotein class contains co-factor activity and this appears related to its protein moiety (4). Since this can now be fractionated into several proteins (5,6) it was of interest to determine whether all or only some of the fractions are involved in lipoprotein lipase activity. This problem was examined with lipid-free and lipid-rich products, the latter obtained by a sonication procedure recently developed in this laboratory (7). The results obtained are the subject of this report.

MATERIALS AND METHODS

Preparation and fractionation of delipidated HDL_2: HDL_2 was separated from whole human

*Operated by the University of Chicago for the United States Atomic Energy Commission.

†Abbreviations: HDL - high density lipoprotein d 1.063-1.21; HDL_2 - subclass of HDL of d 1.063-1.125; apo HDL and apo HDL_2 - delipidated protein moieties of HDL and HDL_2; VLDL - very low density lipoprotein of d < 1.006; LPL - lipoprotein lipase.

serum by ultracentrifugal flotation and was delipidated with 3:2 ethanol:ether at -10° C, as described previously (8). The dried product was re-dissolved in 0.05 M NH_4Cl-NH_4OH buffer, pH 8.3, for immediate use. When sub-fractions of apo HDL_2 were required, the powder was dissolved in 0.05 M Tris-HCl buffer, pH 8.3, containing 8 M urea, and fractionated through Sephadex G200 columns pre-equilibrated with the same buffer (6). The fractions obtained, previously defined as I, III, IV and V (Fraction II was inconstantly seen) were freed from urea by extensive dialysis into the ammonia buffer mentioned above, and their purity was checked by acrylamide gel electrophoresis in 8 M urea.

Relipidation of apo HDL_2 and sub-fractions by sonic irradiation: The method of re-lipidation has been previously described (7). After sonic irradiation, the mixtures of apo HDL_2 (and sub-fractions) and HDL_2 lipids were separated by preparative ultracentrifugation into three products of $d < 1.063$, d 1.063-1.21 and $d > 1.21$, and then dialyzed against the ammonia buffer before use. A similar fractionation procedure was carried out on HDL_2 preparations that had been sonicated under conditions identical to those followed in the re-lipidation experiments.

Preparation and assay of lipoprotein lipase: LPL was purified from human post-heparin plasma by centrifugation of the enzyme-substrate complex followed by detergent treatment and calcium phosphate gel fractionation to remove residual lipoproteins (2,3). The enzyme product was without significant activity against a triolein emulsion in the absence of added lipoproteins. LPL was assayed using the medium previously described (9) except that a radioactive triglyceride emulsion was used as substrate. Glycerol tri-1(^{14}C)-oleate, 33.5 mC/mmole (Radiochemical Centre, Amersham) was diluted with unlabelled triolein (Applied Science Laboratories) in ether to a specific activity of 0.01 mC/mmole, and the triolein was mixed with lecithin (Nutritional Biochemicals, Cleveland) in the proportion 100/6, w/w. After removal of the organic solvent under nitrogen, the lipid was dispersed in 0.145 M NaCl then sonicated in a Branson sonifier (Heat Systems Company, Melville, N.Y.) for three one minute periods at 75 watts, using the standard microtip. Further

periods of sonication up to 10 minutes, or at increased power up to 100 watts, did not increase the rate of enzymatic hydrolysis in the subsequent assay. To this substrate, co-factors in the form of lipoproteins or derivatives were added as described under Results. The unesterified radioactive fatty acid released into the assay medium was measured according to Schotz et al. (10). Sufficient enzyme was added to each ml of assay medium to produce release of 1 μmole fatty acid/hour at 37° C when whole HDL_2 (0.05 mg protein/ml) was the only lipoprotein added.

RESULTS

As shown in Fig. 1, apo HDL_2 permitted a significantly lower maximal rate of enzymatic hydrolysis as compared with the starting product HDL_2. In addition, the activity of apo HDL_2, upon reaching a maximum, decreased as its concentration was raised above 0.01 mg/ml.

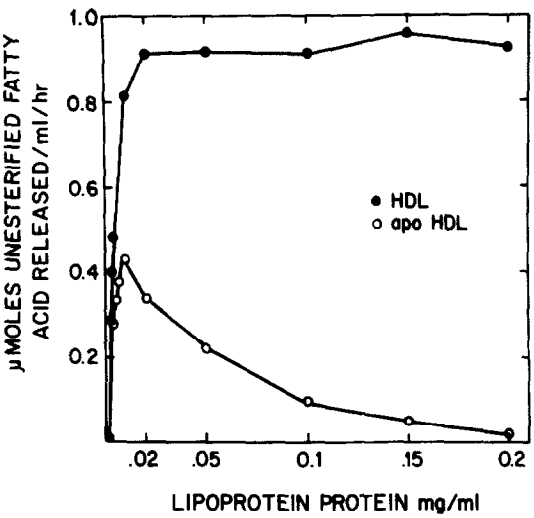

Fig. 1. Effects of HDL_2 and apo HDL_2 upon co-factor activity of LPL. Each assay contained the following: 0.2 ml of sonicated ^{14}C-triglyceride emulsion (10 mg/ml); 0.4 ml of 15% bovine serum albumin in Krebs-Ringer solution, pH 8.3; 0.2 ml of lipoprotein solution (HDL_2 or apo HDL_2) in ammonia buffer; 0.025 ml of enzyme solution in ammonia buffer containing 0.5 mM potassium linolenate (see ref. 2); 0.175 ml of 0.145 M sodium chloride solution. Incubation was for 2 hours at 37° C.

To determine whether each of the protein sub-fractions of apo HDL_2 as separated by gel chromatography had co-factor activity, each was added to the assay system at final

concentrations of from 0.001 to 0.20 mg/ml (Fig. 2). A strong activity was found only with Fraction V. Such activity was essentially absent from Fractions III and IV. Fraction I, which consists of a mixture of all the apo HDL_2 fractions (6), had an activity of 5-10% of that of Fraction V at the same concentration.

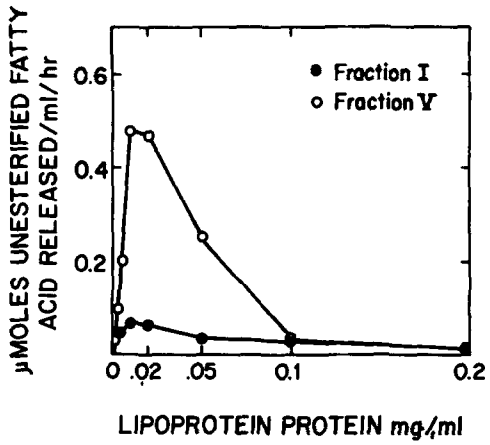

Fig. 2. Cofactor activity of fractions of apo HDL_2. Sephadex fractions in ammonia buffer replaced apo HDL_2 in the assay medium as in Fig. 1.

In view of these results, co-factor activity of sonicated HDL_2 and relipidated apo HDL_2 was investigated. It has been previously shown that after such procedures a moiety rich in Fraction V can be separated by ultracentrifugation (7). Apo-HDL_2 was sonicated with an aqueous suspension of the lipid which had been previously removed from it by extraction with organic solvents. The product was separated by ultracentrifugation into fractions of $d < 1.063$, d 1.063-1.21 and $d > 1.21$ (Fig. 3A). The principal activity was obtained in the $d < 1.063$ cut which contained mainly Sephadex peak V (6). Some activity was found between 1.063-1.21, whereas the material sedimenting at $d > 1.21$ and containing principally Fraction III was totally inactive. Experiments were also carried out with HDL_2 that had been sonicated and fractionated by ultracentrifugation as in Fig. 3A. Very similar results were obtained (Fig. 3B).

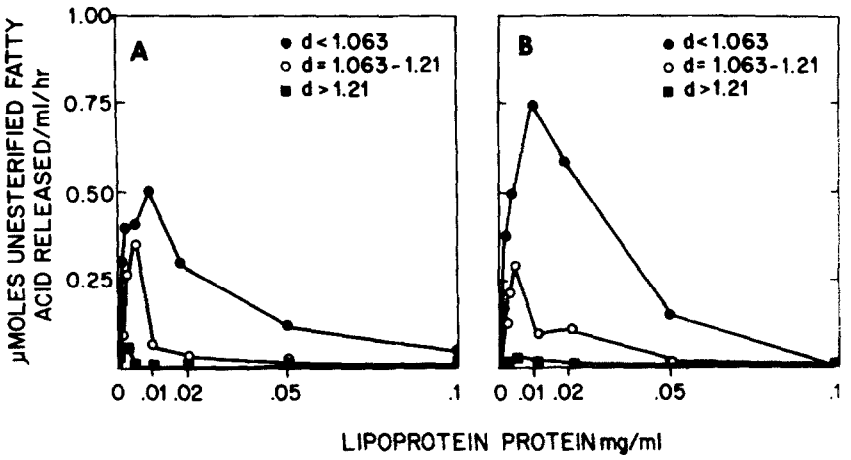

Fig. 3. Co-factor activity of A) sonically irradiated HDL_2 and B)sonicated mixtures of apo HDL_2 and extracted HDL_2 lipid. Experimental details are described in the text. Assay conditions as in Fig. 1.

DISCUSSION

The present results using a purified preparation of LPL from post-heparin plasma confirm previous observations (3,4) that serum HDL* has co-factor activity for this enzyme. They also show that in the system employed, this property is greatly reduced if HDL_2 is replaced by its lipid-free derivative, apo HDL_2 (Fig. 1). The most important aspect of our findings was, however, the observation that essentially all co-factor activity of apo HDL_2 was contained in its Sephadex Fraction V, representing only 5% of this protein (Fig. 2) and containing at least three polypeptide chains of molecular weight about 10,000 (6). The co-factor activity of this fraction was also exhibited by its lipid-enriched forms (Figs. 3A and 3B). We should like to emphasize that the observed similarity in the activity of Fraction V under these different conditions does not necessarily imply that lipid is not required for LPL activation, since phospholipid was a component of our substrate emulsion. Further information on this point may assist in interpreting the differences in the activity

*Such a generalization is justified by the fact that we have found that both subclasses of HDL, HDL_2 and HDL_3 (d 1.125-1.21) exhibit co-factor activity.

curves of HDL_2 and apo HDL_2 observed but not explained by this study. There is evidence that at least some of the polypeptide chains found in apo HDL_2 may be present also in the protein moiety of VLDL (11). It may be relevant that the concentration curve for activation of LPL by VLDL is of the same form as that observed for apo HDL (12).

The elucidation of the importance of such polypeptides in the LPL-mediated degradation of lipoprotein triglyceride will require a better understanding of the structure-function relationships of all protein components of apo-VLDL and apo HDL. Studies in this direction are currently being pursued in this laboratory.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (HE-08727), Life Insurance Medical Research Fund (68-27) and the Chicago and Illinois Heart Associations (RN68-12). C. J. F. is in receipt of Established Investigatorship (#70 142) of the American Heart Association. A. M. S. is in receipt of Research Career Development award (HE-24,867) of the U. S. Public Health Service.

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