LIPOPROTEIN GEOMETRY. I. SPATIAL RELATIONSHIPS OF HUMAN HDL APOPROTEINS STUDIED WITH A BIFUNCTIONAL REAGENT

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SUMMARY

The spatial relationships of the apoprotein components of intact human serum high density lipoprotein particles have been studied using the bifunctional reagent 1,5-difluoro-2,4-dinitrobenzene. The results of crosslinking experiments indicate that the two major HDL apoproteins, apoA-I and apoA-II, lie close to one another in the intact lipoprotein particle. The reproducibility of these results, and the absence of other products signifies that they remain in a relatively fixed position.

INTRODUCTION

In recent years both the lipid and the protein composition of human serum high density lipoprotein (HDL) have been reported in some detail (1) and the primary structures of the two major apoprotein species A-I and A-II are now known (2,3). However, the physical structure and spatial arrangement of these lipid and apoprotein components has yet to be definitively described. Most investigators have approached this problem by utilizing spectroscopic methods, i.e., fluorescence (4), low angle x-ray diffraction (5), NMR (6), etc., techniques which tend to give general rather than specific information on the geometric and topological organization of the HDL components. The major conclusions derived from such studies are that part, at least, of the apoprotein moieties appear to be located on or near the surface of the HDL particle, the phospholipid portions are oriented with their polar heads near the surface, and the more hydrophobic components appear to be more centrally placed (7).

The use of bifunctional reagents for the study of protein geometry is not new (8) and recent applications of the technique have included studies of the

constituent proteins of membranes (9) and ribosomes (10).

There is one report in abstract (11) of the use of the bifunctional reagent suberimidate in a study of human HDL. The data from this report were later interpreted as indicating the linking of A-I to A-I and A-II to A-II (1).

We have employed the chemical crosslinking agent 1,5-difluoro-2,4-dinitrobenzene (DFDNB) as a probe into the nature and extent of interaction of the two major apoprotein components in the intact HDL particle.

MATERIALS AND METHODS

Lipoprotein Preparation: HDL was prepared from fresh human serum by ultracentrifugal flotation as previously described (12). After dialysis against 0.15 M NaCl, containing 10^{-3} M EDTA and 0.02% NaN_3, the HDL was further purified by gel filtration on a Sephadex G-200 column, 2.5 X 100 cm. The resulting protein was stored at 4°C until used, usually within 24 hours.

Crosslinking: The following procedure was chosen after several preliminary experiments. A 1.0 ml reaction mixture consisted of approximately 2 mg HDL protein; 0.15 M NaCl, 10^{-3} M EDTA; 0.025 M $CO_3^=$ buffer, pH 8.8; 20 µg difluorodinitrobenzene. The reaction was permitted to continue at room temperature (22°C) for 6 hours. The reaction was halted by the addition of sufficient 10% sodium dodecyl sulfate (SDS), with or without 10% 2-mercaptoethanol, to an aliquot of the reaction mixture, to give a final SDS concentration of 1%. The resulting suspension was then heated to 60°C for 15 minutes to aid in the solubilization and separation of the apoproteins by SDS.

Gel Electrophoresis: Approximately 50 µg of HDL solubilized as above, was placed on 10 cm, 12% acrylamide gels containing 0.1% SDS, prepared using the procedure of Lammli (13). Electrophoresis was carried out at 1.5 ma/gel, after which the gels were fixed in 12.5% TCA, 40% ethanol, 7% acetic acid; stained with 0.05% Coomassie blue, in 10% ethanol; 7% acetic acid; and destained using a BioRad diffusion destainer. The gels were stored in 7% acetic acid.

Gel Scanning: Gels were scanned using a Beckman Model Acta CII Spectrophotomoter equipped with a GS-2 gel scanner and Beckman Model 1005 Recorder. A slit width of 0.05 mm was used and gels were scanned at 590 nm.

Materials: 1,5-difluoro-2,4-dinitrobenzene and sodium lauryl sulfate were obtained from Sigma Chemicals, St. Louis, Mo. All other chemicals used were reagent grade and were obtained from common suppliers.

RESULTS

Following its reaction with the crosslinking reagent there was no disaggregation of the HDL particle as evidenced by analytical ultracentrifugation.

Scans of the SDS gels resulting from a typical crosslinking experiment using intact human HDL particles are shown in Figure 1. Figure 1a, the scan of the electrophoresis gel of the control HDL solution, indicates the presence of

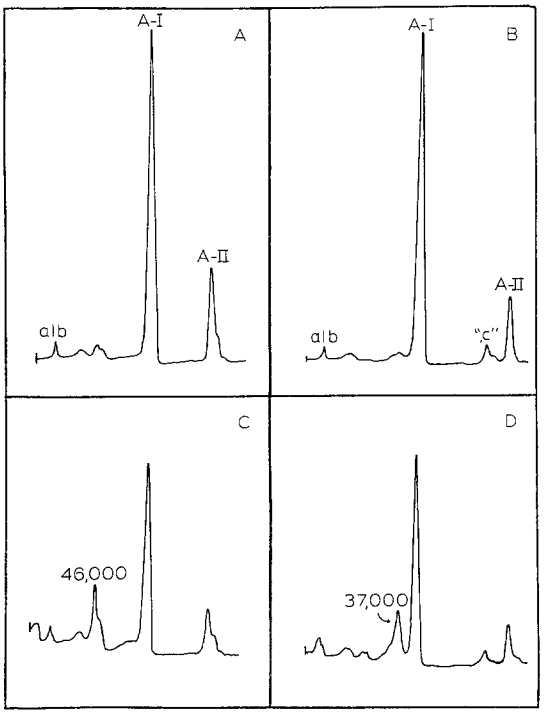

Fig. 1. SDS polyacrylamide gel electrophoresis of SDS solubilized apoproteins of HDL. (a) Control, without reducing agent. (b) Control, with 1% 2-mercaptoethanol. (c) DFDNB-crosslinked HDL, no reducing agent. (d) DFDNB-crosslinked HDL, with 1% 2-mercaptoethanol.

four quantitatively significant protein species: A protein of MW 68,000 (presumably albumin), apoA-I (MW 28,300), apoA-II dimer (MW 17,500), and the C-peptides. Figure 1b shows the scan of the HDL control if 1% 2-mercaptoethanol is added to the solubilization solution. The A-II dimer has been split into monomeric units of MW 8,750 each. Figures 1c and d show the scan of crosslinked HDL. In 1c the only significant new peak appears at a MW of approximately 46,000. Figure 1d is the scan of crosslinked HDL in the presence of 1% 2-mercaptoethanol. The major peak has been shifted to MW 37,000. As can be seen from Figs. 1c and d, these are the major crosslinking products. There are no significant peaks corresponding to products of crosslinking two or more A-I or two or more A-II units. The molecular weights were determined from the recorded scans using added MW markers as well as the location of albumin and apoproteins of known MW.

DISCUSSION

Considering only apoprotein A-I (MW 28,330) and apoprotein A-II (a disulfide dimer of MW 17,500) the appearance of a crosslinked product with MW of 46,000 could only be the result of one A-I linked to one A-II unit. This conclusion is supported by Fig. 1d which shows that the addition of the disulfide cleaving agent, 2-mercaptoethanol, results in the loss of half of the A-II dimer leaving a product consisting of one A-I linked to one A-II monomer whose MW would be approximately 37,000.

From our data we conclude that at least some of these two apoprotein components lie very close to one another in the intact lipoprotein particle since the crosslinking reagent will only crosslink groups less than 10 Å apart. If each HDL particle contains both A-I molecules and A-II molecules, even though the exact molar ratio, if there is one, is not yet agreed upon (1), a number of possible crosslinking products exists. From our results with the 5-10 fold molar excess of DFDNB used in these experiments, several additional conclusions seem probable. First, the major crosslinked product appears to be one A-I plus one A-II; second, there is no indication of any A-II intramolecular crosslinking which might be expected with a molecule already crosslinked by disulfide bridges; third, A-I is bound to only one of the A-II units in the A-II dimer. Also, there is no evidence for significant association of the two A-I apoprotein chains as suggested by Scanu in work with another reagent (1). Since both apoA-I and apoA-II contain numerous lysine residues, fairly evenly distributed throughout the molecules (2,3), any close association of the chains should produce multiple sites for possible crosslinking. There is no evidence for participation of "C" peptides in the crosslinking.

Experiments using higher molar ratios (10-100X) of crosslinker were carried out as described above. Although many apparently high molecular weight products were observed, under no conditions tested did the gels indicate significant A-I - A-I crosslinking or A-II intramolecular crosslinking.

The reproducibility of these crosslinking experiments on HDL obtained from several donors suggests that in the intact HDL particle the apoprotein

components may be found in a rather fixed spatial orientation to one another.

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