LIPOPROTEIN GEOMETRY. II. APOPROTEIN EXCHANGE IN HUMAN PLASMA HIGH DENSITY LIPOPROTEIN

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SUMMARY

The relationships between the apoproteins of intact human serum high density lipoprotein particles, HDL_2 and HDL_3, have been studied by observing the exchange of radioactively labeled apoproteins between one subclass and the other. This exchange process can be inhibited by chemically crosslinking the apoproteins of either the labeled or unlabeled subclass. These results are consistent with a dynamic relationship between HDL_2 and HDL_3 which appears dependent upon the association and perhaps the conformation of the apoprotein components of the lipoprotein particles.

INTRODUCTION

Human plasma high density lipoprotein (HDL)[1] is commonly divided into two subclasses, HDL_2 and HDL_3. These subclasses differ in their buoyant density and molecular weight, but contain essentially the same protein and lipid components (1). Whether or not HDL_2 and HDL_3 represent distinctly different functional and/or structural particles or even collections of different particles is still an open question.

Recent experiments by Friedberg and Reynolds (2) suggest that the molar ratios of the major apoproteins of HDL [A-1 (ApoGln-I) and A-2 (ApoGln-II)] remain constant from individual to individual as well as from subclass to subclass. In our laboratory we have shown that the crosslinking patterns obtained using a variety of bifunctional reagents suggest that A-1 and A-2 are located in close proximity in both HDL_2 and HDL_3 and, additionally, have presented evidence which indicates that for HDL_2 and HDL_3, the crosslinking patterns are

[1]Abbreviations used: HDL, high density lipoprotein; DFDNB, 1,5-difluoro 2,4-dinitrobenzene; FDNB, 1-fluoro 2,4-dinitrobenzene; BSA, bovine serum albumin, SDS, sodium dodecyl sulfate

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essentially the same (3,4). Such information implies that at least some portions of the structure of these lipoprotein particles remain in a relatively fixed spatial arrangement for significant periods. If the ratio of A-1 to A-2 dimer is 2:1, in both HDL_3 and HDL_2 as suggested by Friedberg and Reynolds (2), then the difference between HDL_2 and HDL_3 may represent the addition of one A-1:A-2 unit, which consists of two A-1 peptides plus one A-2 peptide dimer. As this additional protein does not appear to be crosslinked to the other A-1:A-2 unit (4), it is necessary to consider possible modes of association of the apoproteins of the two units proposed for HDL_2.

To investigate this possible association, as well as the relationship between the apoproteins of the different classes of HDL, we have examined the exchange of labeled apoproteins between HDL_2 and HDL_3, in the presence and absence of bifunctional crosslinking reagents.

MATERIALS AND METHODS

Materials: [^{125}I] was purchased from New England Nuclear as [^{125}I]-NaI in NaOH solution. 1,5-difluoro 2,4-dinitrobenzene (DFDNB) and 1-fluoro 2,4-dinitrobenzene (FDNB) were purchased from Sigma Chemicals, St. Louis, Mo. All other chemicals used were reagent grade and were obtained from common suppliers.

SDS Polyacrylamide Gel Electrophoresis: 7.8% acrylamide gels were prepared and run using the method of Fairbanks (5). The gels were either fixed in 40% propanol in 7.5% acetic acid and stained with Coomassie Blue or sliced into 2mm slices using a Bio-Rad Gel Slicer.

Lipoprotein Preparation: HDL was prepared from human serum by ultracentrifugal flotation as previously described (6). After dialysis against a KBr-NaCl solution (d=1.125 gm/cc, 0.15M NaCl, 10^{-3}M EDTA, and 0.02% NaN_3), HDL_2 and HDL_3 were separated by ultracentrifugation for 36 hrs at 45,000 RPM in a Beckman Ti 60 rotor. The lipoprotein subclasses were removed using a Pasteur pipette, then dialyzed against 0.15M NaCl containing 10^{-3}M EDTA and 0.02% NaN_3, and stored at 4°C until use. Protein concentrations were determined by the method of Lowry (7) with BSA as a standard.

Iodination of Lipoproteins: 5.0 ml of the sample to be labeled was dialyzed against 0.5M glycine buffer, pH 9.4 containing 0.15M NaCl, 10^{-3}M EDTA, 0.02% NaN_3, and then labeled using the iodine monochloride method (8). 0.5 ml of iodine carrier solution containing 250 µCi of [^{125}I]-NaI was added to the HDL solution with mixing. After 10 minutes the unreacted iodine and glycine buffer were removed by column chromatography using Sephadex G-25 equilibrated with 0.15M NaCl containing 10^{-3}M EDTA and 0.02% NaN_3. Protein solutions with a specific activity of 5,000 to 10,000 cpm/µg were routinely obtained using this method. Unlabeled controls were treated as above except for the addition of the [^{125}I]-iodine carrier solution. It could be routinely shown that approximately 99.8% of the radioactivity was associated with the apoproteins while only 0.2% was found in the lipid fraction.

Crosslinking and Monofunctional Labeling with FDNB: 0.25M Na_2CO_3 buffer, pH 8.8, was added to the protein solution to be modified to achieve a final concentration of 0.025M Na_2CO_3. DFDNB or FDNB dissolved in ethanol was then added with rapid mixing to give the desired molar ratio of crosslinker or FDNB to protein. The reaction was allowed to proceed for two hours at room temperature. Experiments in our laboratory have demonstrated that increasing amounts of DFDNB produce increasing amounts of high molecular weight crosslinked products in HDL without disruption of the HDL particle (3,4). We have also shown that FDNB binds covalently to the protein portion of HDL as does DFDNB. The reaction mixture was used directly without further treatment as it could be shown that only very small amounts of the free hydrolysis products of DFDNB or FDNB were formed.

Incubation of HDL_2 and HDL_3 Mixtures: The desired amounts of labeled and unlabeled HDL_2 and HDL_3 in solution were added directly to 5/8" x 3" cellulose nitrate centrifuge tubes and the tubes placed in a 37°C constant temperature water bath for 6-7 hours. SDS - polyacrylamide gel electrophoresis of controls showed no apparent differences between incubated and unincubated HDL_2 or HDL_3 suggesting that bacterial action was not significant. For all the incubations described in this paper the following volumes and concentrations were used: Controls - 200 µl containing 300 µg of HDL protein, Mixtures - 400 µl containing 300 µg labeled HDL protein and 300 µg cold HDL protein. Results obtained from experiments using more dilute incubation solutions were very similar to those produced from the conditions described above. For the chaotropic ion experiments, solid KBr was added directly to the solutions to attain the desired concentration of Br^- ion.

Separation of Reacted HDL_2 and HDL_3: Sufficient KBr-NaCl solution (d=1.3245 gm/cc containing 0.15M NaCl, 10^{-3}M EDTA, 0.02% NaN_3) was added to the reaction mixture to give a final density of d=1.125 gm/cc. KBr-NaCl solution d=1.125 gm/cc was then added to give a final volume of 10 ml. Each tube was then underlayered with 1.0 ml of d=1.21 gm/cc KBr-NaCl solution, capped and placed in a Beckman Ti 50 rotor. The samples were centrifuged at 42,000 RPM for 36-48 hrs. Thirty drop (0.6 ml) fractions were collected from each tube after piercing the bottom of the tube. The fractions were then counted and the protein concentration determined by the Lowry method (7) or by reaction with fluorescamine (9).

Determination of Radioactivity: All counting of [^{125}I] was done using a Nuclear Chicago Model 8725 manual gamma counter. The counting efficiency was approximately 52%. All samples were counted in 13 x 100 mm glass tubes using the same volume of solution in each tube.

RESULTS

Figs. 1A and 1B show the ultracentrifugal fractionation pattern obtained for controls of labeled HDL_2^* and HDL_3^* incubated in the absence of any other protein. The data is given in counts per min. per fraction as it was found that the radioactivity and protein concentration curves coincided (the specific activities of control fractions were constant throughout the tube). Fig. 1 also illustrates the results obtained after mixing and incubation of equal amounts of labeled HDL_3^* and cold HDL_2 (1A) or labeled HDL_2^* and cold HDL_3 (1B).

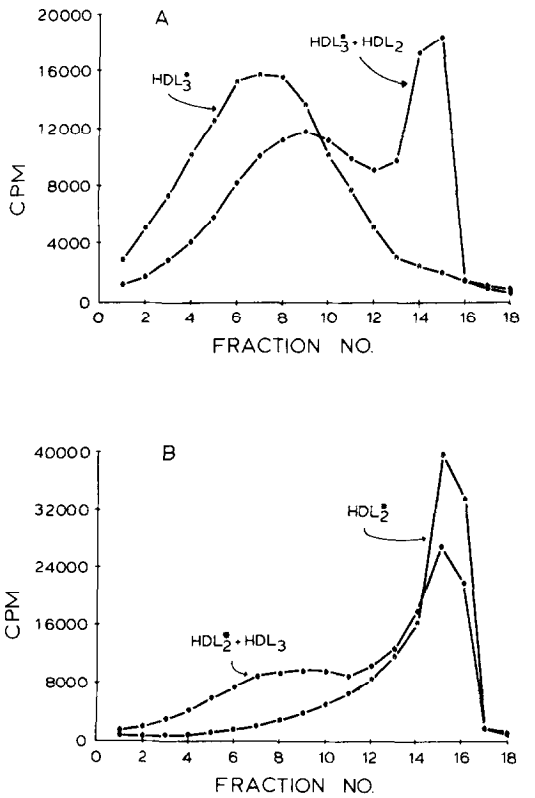

Fig. 1. Distribution of label after centrifugation to separate HDL_2 and HDL_3. *indicates labeled subclass. A. HDL_3^* control; HDL_3^* after incubation with cold HDL_2. B. HDL_2^* control; HDL_2^* after incubation with cold HDL_3.

In Fig. 1A it can clearly be seen that a transfer of label from HDL_3 to HDL_2 has occurred. Further, the specific activity of the HDL_3 fraction was significantly decreased when compared to controls. In a typical experiment, the values for a selected fraction in the HDL_3 region of the tube (fraction 6) were: HDL_3^* control = 2850 cpm/μg; HDL_3^* incubated with HDL_2(cold) = 1680 cpm/μg. Similar results were obtained when labeled HDL_2^* was incubated with cold HDL_3 (Fig. 1B). However, due to the breadth of the HDL_3 peak as compared with the HDL_2 fractions the results are not easily visualized. It was observed that when incubations were carried out in the presence of 2.0M Br^- ion, the amount of exchange in a given period of time was increased by about 30% over mixtures containing only

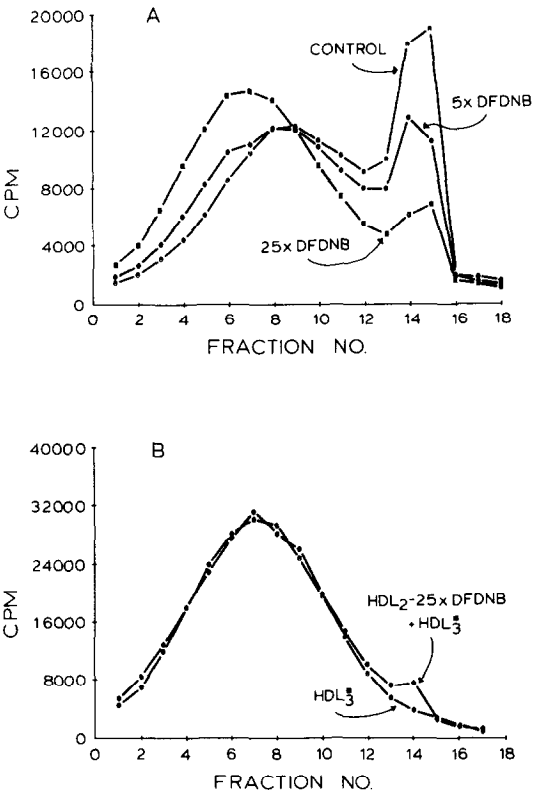

Fig. 2. Distribution of label after centrifugation to separate HDL_2 and HDL_3. A. HDL_3^* incubated with HDL_2; HDL_3^* crosslinked with a 5 fold molar excess of DFDNB followed by incubation with HDL_2; and HDL_3^* crosslinked with a 25 fold molar excess of DFDNB then incubated with HDL_2. B. HDL_3^* control; HDL_3^* incubated with HDL_2 which was crosslinked with a 25 fold molar excess of DFDNB.

0.15M NaCl solution. This increased exchange could be demonstrated regardless of which subclass was initially labeled.

If the labeled HDL_3^* is crosslinked with DFDNB before incubation with HDL_2, the transfer of labeled protein is reduced depending upon the extent of crosslinking. Fig. 2A shows the three fractionation patterns obtained with: [^{125}I]-HDL_3^* incubated with HDL_2 (control); [^{125}I]-HDL_3^* crosslinked with a 5 fold molar excess of DFDNB (3) and then incubated with HDL_2; [^{125}I]-HDL_3^* incubated with HDL_2 following crosslinking the HDL_3^* with a 25 fold molar excess of DFDNB. It can be seen that increased crosslinking results in decreased exchange. To rule

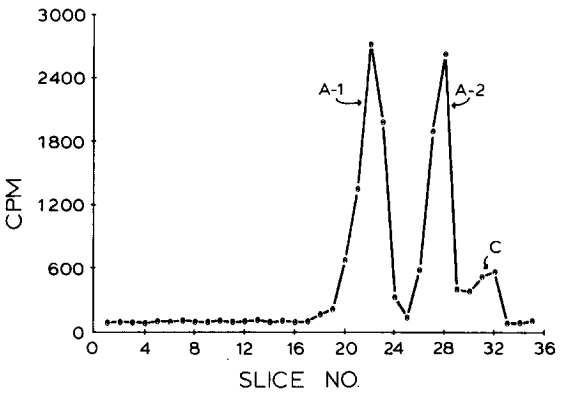

Fig. 3. SDS-PAGE of HDL_2 after incubation with labeled HDL_3^* followed by reseparation. The gel was sliced into 2mm slices and the slices counted.

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out the possibility that the monofunctional substitution of the apoproteins with the crosslinking reagent was the cause of this decrease in exchange, FDNB, a monofunctional reagent, was used to modify the proteins. Reaction with concentrations of FDNB, comparable to the amounts of DFDNB that greatly inhibited exchange, had little effect on the exchange process. Fig. 2B shows the results obtained when labeled HDL_3^* was incubated with cold HDL_2 that had been crosslinked with a 25 fold molar excess of DFDNB. The exchange is also inhibited as it was with the crosslinked HDL_3^*.

Gel electrophoresis of the recipient HDL subclass, into which labeled apoproteins have been transfered, indicates that A-1, A-2, and apparently the C-peptides are all exchanged. Fig. 3 shows the radioactivity pattern obtained when HDL_2, into which labeled apoproteins have been transfered by incubation with $[^{125}I]$-HDL_3^*, is run on SDS polyacrylamide gel electrophoresis. The radioactive bands were identified by staining and scanning duplicate gels.

DISCUSSION

The transfer of labeled apoprotein from one HDL subclass to another does not proceed with a loss of protein content of the originally labeled fraction. The specific activity of that fraction decreases but the protein content remains

unchanged. This is consistent with an exchange process in which there is a one to one exchange of protein of one subclass with the other. Such exchange could occur by the transfer of single apoprotein polypeptides, i.e., A-1 or A-2; or by the exchange of complexes of apoproteins such as 2A-1:1A-2. Our results do not permit us to rule out either of these possibilities. It might be suggested that since crosslinking inhibits the exchange, single polypeptide chains are involved. However, the crosslinking may also prevent a conformational change that might be necessary for the process. That a conformational change might be involved is suggested by the fact that when high concentrations of the chaotropic ion, Br^-, are included in the incubation mixture, increases in the rates of exchange are observed [chaotropic ions are known to affect protein interactions and structure (10)]. An actual exchange involving a conformation change is also suggested by the finding that crosslinking the unlabeled subclass also greatly inhibits the exchange process. If a simple transfer of peptide into the recipient class occurred, one would not expect crosslinking to have much effect.

If HDL_2 is made up of two identical subunits as suggested by Friedberg and Reynolds (2), the transfer of label might be visualized in terms of the exchange of HDL_3 for one of the HDL_2 subunits. Although this is a reasonable possibility, one must again consider the possibility of a conformational change to explain the results of the crosslinking experiments.

From this data we conclude that there is a dynamic relationship between HDL_2 and HDL_3. Even though these two subclasses can exist as stable separate entities, when they are present together in solution, significant interaction between particles may occur. Exchange of apoproteins could possibly occur between HDL_2-HDL_2 or HDL_3-HDL_3 as well as exchange between HDL_2 and HDL_3 molecules. Studies are now underway to determine the relationship between the protein exchange described herein and any possible exchange of lipid.

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