papers and notes on methodology

Loss of human serum apolipoproteins C and E during manipulation of diluted solutions

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Abstract The adsorption of human serum very low density apolipoproteins C-I, C-II, C-III, and E to the interior surfaces or Beckman Airfuge plastic tubes and glass pipettes has been studied. ^{125}I-Labeled apolipoprotein C-III-1 (1 μg/ml) was used as a model compound to study the adsorption. After 60 min about 45% was bound to the tube walls, and 5–10% bound rapidly to the pipette used for removing the solution from the tube. Addition of increasing amounts of non-labeled apolipoprotein C-I, C-II, C-III, or E to the tubes reduced the amount of labeled C-III on the tube walls, indicating a strong affinity of these apolipoproteins for the surface.

The results demonstrate the importance of careful control of the different steps in immunological ultramicroquantitation methods, that might be influenced by adsorption-losses of the apolipoproteins.—**Holmquist, L.** Loss of human serum apolipoproteins C and E during manipulation of diluted solutions. *J. Lipid Res.* 1982, **23:** 357–359.

Supplementary key words albumin • heparin • Intralipid® • VLDL

Delipidated human serum apolipoproteins might be expected to adsorb very strongly to glass and plastic surfaces (1). Adsorption may exert a most serious influence on the preparation of primary standard solutions of purified apolipoproteins to be used in immunological ultramicroquantitation methods. The adsorption process may occur in minutes (2) on vessels, test tubes, and pipettes used in protein determinations of stock solutions to be diluted to working standards, yielding nominal concentration values that may largely deviate from the true ones.

The present communication reports the results from a study of the adsorption of isolated human serum apolipoproteins C-I, C-II, C-III, and E to the interior of Beckman Airfuge plastic tubes and to glass pipettes used during manipulation of diluted solutions of the apolipoproteins. The increasing use of sensitive immunological quantitation methods of apolipoproteins (3), utilizing solutions with a working standard range of about 5–200 ng protein per ml, motivated this presentation.

Abbreviation: VLDL, very low density lipoprotein d < 1.006 g/ml.

MATERIALS AND METHODS

Beckman Airfuge tubes (cellulose nitrate) were obtained from Beckman Instrument, Inc., Palo Alto, CA. VLDL and sodium chloride solution d 1.006 g/ml were prepared as described by Lindgren, Jensen, and Hatch (4). Intralipid® (10%, diluted to 1% with sodium chloride solution, d 1.006 g/ml) and purified heparin were bought from Vitrum, Stockholm, Sweden.

Human serum apolipoproteins C-I, C-II, C-III-1, and E were isolated from VLDL by selective extraction with organic solvents (5) and purified as described previously (6–8). Apolipoprotein C-III-1 was labeled with ^{125}I and purified on Sephadex G-25 using the sodium monochloride method of McFarlane (9). The protein fraction from the column was further dialyzed against 0.15 M sodium chloride, yielding a product that contained less than 1% free iodine. The specific activity of the labeled apolipoprotein was 150×10^6 cpm/mg.

Radioactivity was determined in a Packard Autogamma scintillation spectrometer 5230 (Packard Instrument Co., Inc., Downers Grove, IL).

PROCEDURE

The experiment to study the adsorption of the apolipoproteins was designed in the following way. A Beckman Airfuge tube (0.30 ml capacity) was filled with 165 μl of sodium chloride solution (d 1.006 g/ml). To this solution 200 ng of ^{125}I-labeled apolipoproteins C-III-1 in 10 μl of sodium chloride solution was added. The addition of labeled protein was made by injection from a microliter glass syringe after first rinsing the syringe several times with the protein solution. The content of the tube was mixed by the syringe needle. After 60 min at room temperature, the contents of the tube were removed with a glass Pasteur pipette and transferred within 30 sec to a counting vial. The tube was then filled six times with 0.30 ml of sodium chloride solution and emptied each time with the same Pasteur pipette used for removal of the original solution from the tube. The washings were combined with the protein solution in the counting vial.

The washed Beckman tube was placed in a second vial and the part of the Pasteur pipette that had been in contact with the radioactive solution and subsequently washed was cut off, crushed, and placed in a third counting vial.

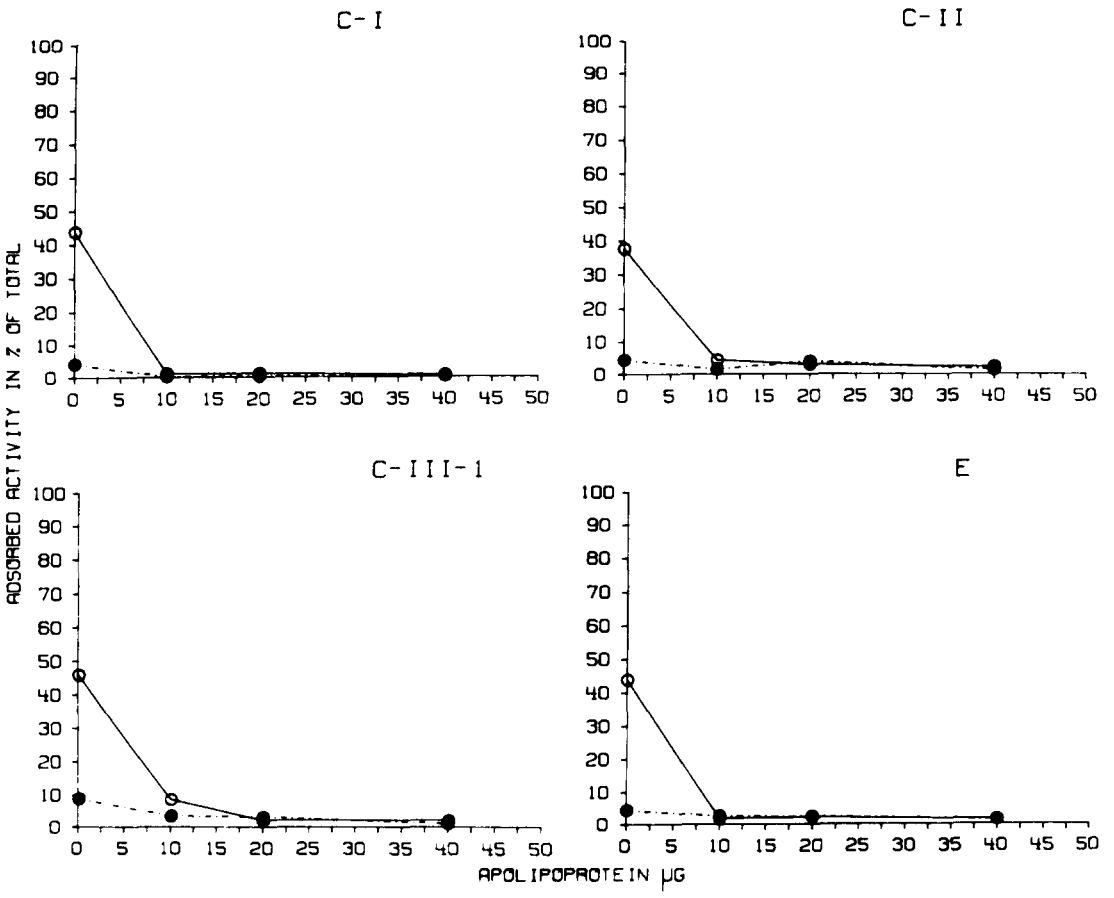

Fig. 1. Ability of non-labeled apolipoproteins C-I, C-II, C-III-1, and E to inhibit the adsorption of ^{125}I-labeled apolipoprotein C-III-1 to the interior surface of Beckman Airfuge plastic tubes (O, solid lines) and to glass Pasteur pipettes (●, dashed lines). Each tube contained 10 µl of a solution of labeled C-III-1 and 165 µl of sodium chloride solution (d 1.006 g/ml) of the apolipoprotein to be tested. Incubation was performed for 60 min at room temperature.

In order to study the affinity of non-labeled purified apolipoproteins and lipids for the solid surfaces, each substance was dissolved in sodium chloride solution and added in increasing amounts to the tubes. After adjusting the volume in each tube to 165 µl with sodium chloride solution, 10 µl of radiolabeled apolipoprotein C-III was added and the tubes were left for 60 min as described above. The distribution of radioactivity between tube, pipette, and solution was determined as described above, for non-labeled apolipoproteins C-I, C-II, C-III-1, and E, and intact VLDL, Intralipid,® heparin, and bovine serum albumin.

RESULTS AND DISCUSSION

Approximately 200 ng of apolipoprotein C may be calculated to theoretically bind as a monomolecular layer to a surface area of one cm^2.

Radiolabeled apolipoprotein C-III-1 was chosen as a model compound to study adsorption and it bound to the extent of about 45% (90 ng) after 60 min to the tube walls. Between 5 and 10% bound rapidly to the pipette used for removing the solute from the tube, in the absence of other proteins.

Addition of increasing amounts of non-labeled apolipoproteins C-I, C-II, C-III-1, or E proportionally reduced the adsorption of labeled C-III-1 (**Fig. 1**), demonstrating a strong affinity of all apolipoproteins for the plastic and glass surfaces.

The displacement of radiolabeled C-III-1 from tubes and pipettes by non-labeled C-III-1 indicates that the ^{125}I-labeled apolipoprotein per se was not solely responsible for the adsorption phenomenon. The efficiency of the different apolipoproteins to compete with the labeled C-III-1 for the solid surfaces seems to be in the order C-I > C-II > C-III-1 in the present experiments.

The affinity of the apolipoproteins to the surfaces was found to be much stronger than that of bovine serum albumin (**Fig. 2**). At an albumin concentration of 0.1%, 15% of the radioactivity was still bound to the tube walls. The polysaccharide heparin, which has been demonstrated to interact with intact lipoproteins, was not able to desorb the labeled C-III-1 (Fig. 2).

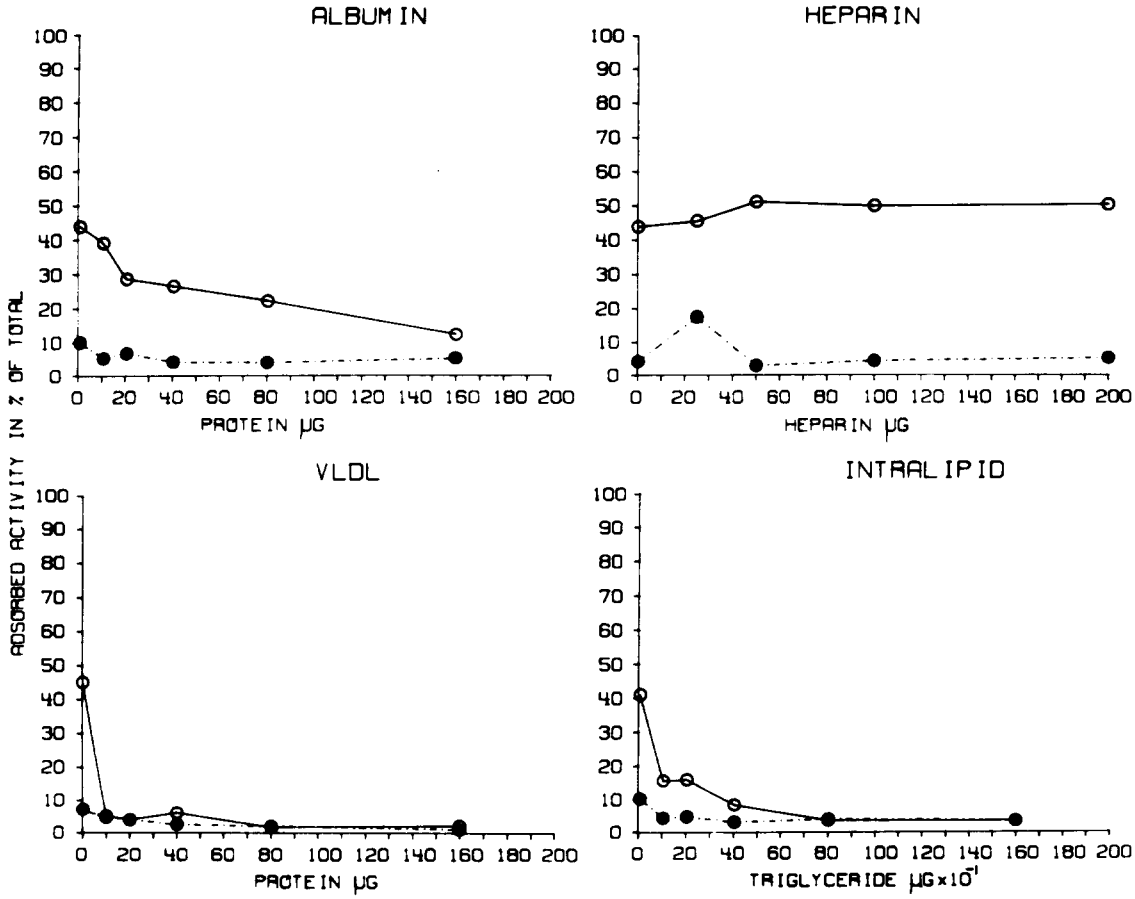

Fig. 2. Ability of albumin, heparin, VLDL, and Intralipid® to inhibit the adsorption of ^{125}I-labeled apolipoprotein C-III-1 to the interior surface of Beckman Airfuge plastic tubes (O, solid lines) and to glass Pasteur pipettes (●, dashed lines). Incubation conditions as in Fig. 1.

Native VLDL and Intralipid® (emulsified triglycerides) seemed effective in desorbing surface-bound radioactivity (Fig. 2). However, this effect may be secondary to the binding of labeled apolipoprotein C-III-1 to the lipid-containing particles, which might be stronger than the binding to solid surfaces. This experiment can not discriminate between these two possibilities.

In view of the present analysis, which demonstrates a strong and rapid adsorption of apolipoproteins C and E to glass and plastic surfaces, it seems necessary to carefully control the different steps in apolipoprotein quantitation methods that might be influenced by losses of the apolipoproteins due to adsorption.

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