IMMUNOCHEMICAL DETECTION OF APOPROTEIN DISSOCIATION FROM VERY LOW DENSITY LIPOPROTEIN PARTICLES IN HUMAN BLOOD PLASMA

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UDC 616.153.96:577.112.856]-074:543.544

KEY WORDS: very low density lipoprotein; apolipoprotein E; apolipoprotein C-II; apolipoprotein C-III; dissociation of apoproteins

Very low density lipoproteins (VLDL) are the main carriers of endogenous plasma triglycerides. They contain apoproteins B and E, and of the C group. ApoE and apoC belong to the class of amphipathic proteins and are in a state of equilibrium between the lipid interphase and the aqueous medium. ApoE in the composition of lipoproteins is known to be a ligand of cellular apoE- and apoB,E-receptors and to be involved in the clearance of remnants of triglyceride-enriched lipoproteins. The apoC group includes an activator of lipoprotein lipase, the key enzyme of lipid metabolism in extrahepatic tissues, as well as apoC-II and its inhibitor apoC-III. The writer showed previously that treatment of VLDL with detergent or lipoprotein lipase in vitro leads to considerable dissociation of apoE and apoC from lipoprotein particles [2]. The process of apoprotein dissociation may be important for the regulation of lipid metabolism in vivo and it may also affect the results of determination of human serum apoproteins under detergent-free conditions.

The aim of this investigation was to detect dissociation of apoproteins from native VLDL at below physiological concentrations with the aid of antibodies (AB).

EXPERIMENTAL METHOD

The apoE preparation was obtained from human blood plasma by the method in [1] and apoC-II and apoC-III_1 were obtained by the method in [7]. The proteins were labeled with fluorescein isothiocyanate (FITC) by the method in [13]. Antisera to each apoprotein were obtained by primary immunization of rabbits with 0.5 mg apoprotein with Freund's complete adjuvant, and in two repetitions, with incomplete adjuvant. The apoproteins were cross-linked to CNBr-activated sepharose by the method in [4] in buffer containing 5 mM Na cholate. Monospecific polyclonal AB were isolated by immunoaffinity chromatography of antisera on the corresponding apoprotein-sepharose. Fab-fragments of AB to each apoprotein were obtained by treatment with papain [14]. Binding of FITC-labeled apoproteins with Fab-fragments of monospecific AB to apoC-II and apoC-III_1 were studied by measuring the change in anisotropy of fluorescence of FITC residues according to the method in [6]. The dissociation constant and stoichoimetry of binding of VLDL with AB for each apoprotein was estimated by separating the bound and free forms of FITC-labeled Fab-fragments of AB by gel-chromatography on Toyopearl HW-60F (1.6 × 51), with detection according to fluorescence of FITC and analysis of the results by Scatchard plot [12]. Protein was determined by the method in [8] in the presence of 5% SDS. Theoretical analysis of the saturation fraction of apoprotein on the surface of a stabilized lipoproteinlike microemulsion of ovolecithin/triolein was calculated by the equation:

$$p_B/B_O = (p_T + K + B - (p + K + B_O)^2 - 4pB_O)^{1/2}/2B_O \quad (1)$$

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 11, pp. 485-487, November, 1992. Original article submitted April 21, 1992.

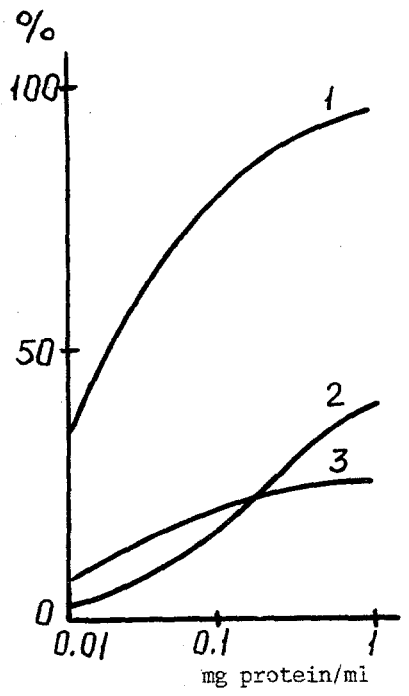

Fig. 1

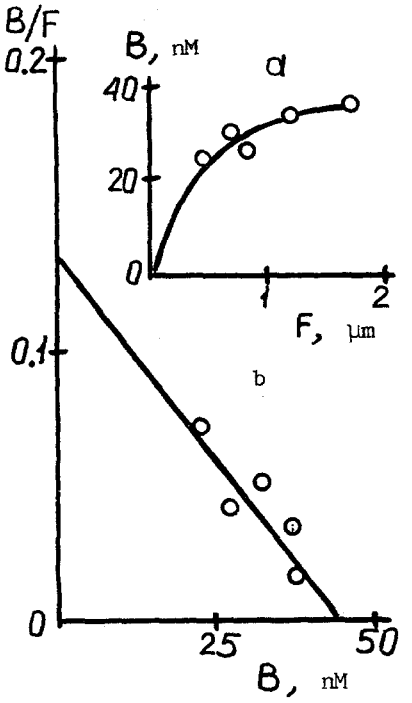

Fig. 2

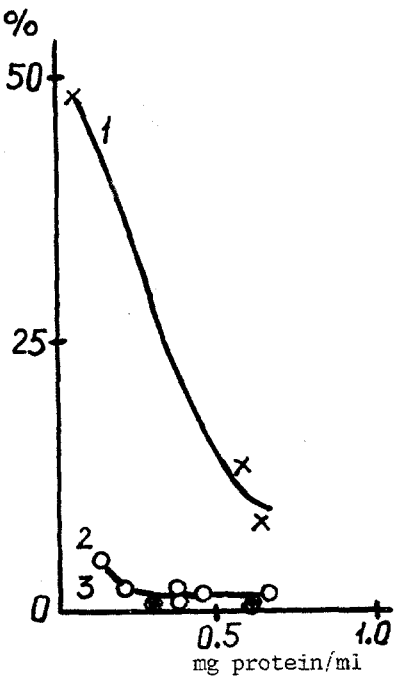

Fig. 3

Fig. 1. Dependence of theoretically calculated saturation of lipid surface with apolipoproteins C-III (1), E (2), and C-II (3) on VLDL protein concentration.

Fig. 2. Binding of Fab-fragments of antibodies to apolipoprotein C-II with VLDL: a) saturation curve, b) Scatchard plot. A typical experiment is shown.

Fig. 3. Dependence of stoichiometry of binding of Fab-fragments of antibodies to apolipoprotein with apoE (1), apoC-II (2), and apoC-III (3, filled circles) in composition of VLDL on VLDL protein concentrations in preparation.

where p_B denotes the concentration of bound protein, $B_0 = B_S \cdot pl$ (where B_S is the level of saturation of the phospholipid surface with the apoprotein, pl denotes the phospholipid concentration), p_T is the total concentration of apoprotein, K_D the dissociation constant of the apoprotein/phospholipid complex [11]. The following assumptions and known parameters were used in the calculations: concentration of phospholipids and protein in VLDL 18 and 10% respectively, mean molecular mass of phospholipid 800 daltons, concentration of apoC-II, C-III, and E in VLDL 6, 7, 40, and 13% respectively [3].

EXPERIMENTAL RESULTS

Theoretical analysis of dissociation of apoproteins E and C from the surface of VLDL particles was carried out on the basis of two hypotheses: the whole pool of phospholipids on the surface of the particles is potentially accessible for binding with these apoproteins; binding of one apoprotein does not affect binding of another, i.e., there is an excess of binding sites. On the basis of these assumptions and known parameters of binding and of the apoprotein content in VLDL the degree of dissociation of apoE, C-II, and C-III from the surface of VLDL was calculated during dilution of the VLDL particles (Fig. 1). Thus during work with dilute solutions of VLDL the degree of dissociation may reach appreciable values. For experimental confirmation of the suggested behavior, the

immunochemical reactivity of the apoproteins was studied. Preliminary experiments with binding of Fab-fragments of antiapoproteins with purified FITC-labeled apoproteins in solution showed good affinity of the AB obtained. K_D of the antigen–antibody complex in solution was 12 nM for apoC-II and 20-75 nM for apoC-III, with stoichiometry close to 1. The study of binding of AB to apoE, C-II, or $C\text{-}III_1$ with VLDL was carried out by chromatographic separation of AB bound and not bound with VLDL. The saturation curve (a) and Scatchard plot (b) of a typical experiment to study binding of Fab-fragments and VLDL are illustrated in Fig. 2. Analysis of dependence of the stoichiometry of binding of AB and VLDL, shown in Fig. 3 was carried out with allowance for the concentrations of apoE, C-II, and C-III in VLDL indicated previously. It was found that dilution of the VLDL preparations led to a marked increase in accessibility of the antigenic determinants of apoE: from 7% with a protein concentration of VLDL of 0.6 mg/kg to 48% at 0.77 mg/ml (Fig. 3, curve 1). Meanwhile, dilution of VLDL had only a very slight effect on the increase in binding of Fab-fragments of antiapoC-II with VLDL; from <1% at a VLDL concentration of 0.4-0.6 mg/ml to 4.8% at 0.2 mg/ml of VLDL (Fig. 3, curve 2). Accessibility of apoC-III on the surface of VLDL for AB, however, was extremely low (<0.15%) and was virtually independent of the VLDL concentration within the working range (Fig. 3, curve 3). On the other hand, complete exposure of the antigenic determinants of apoC-III on the surface of the lipoprotein particles is suggested in [9]. However, in the work cited, VLDL- with a protein concentration of 0.3-15 μg/ml was used, which evidently corresponds to a high degree of dissociation of apoC-III from the lipoprotein particle. Experiments on binding of FITC-labeled Fab-fragments with VLDL, studied by measuring the change in anisotropy of fluorescence of FITC residues, showed no effect of VLDL on the anisotropy of FITC-labeled Fab-fragments. We suggested that the low VLDL concentration used in these experiments (1-5 μg protein/ml) corresponds to virtually complete dissociation of the amphipathic apoproteins apoE, C-II, and C-III from the lipoprotein surface. It can thus be concluded that accessibility of the amphipathic apoproteins on the surface of VLDL for AB is very low, and that dilution of the VLDL preparation increases the accessibility of apoE and apoC-II, confirming the results of theoretical analysis.

The results of our previous investigation of quenching of fluorescence of apoVLDL suggested that apoproteins of VLDL form a cluster on the surface of the particle [2]. It follows from these data that dissociation of apoproteins from the surface of the particle leads to separation at least of apoproteins E and C-II from clusters on the surface of the particle and to an increase in their accessibility for AB. The results also are in agreement with [10], where immunochemical reactivity of only 15% of apoA-I was observed in native high-density lipoproteins. An investigation [5] established that on the addition of detergent to whole plasma and during lipolysis of VLDL particles there is an increase in the degree of exposure of the apoC-II epitopes. We suggest that the process of dissociation can be stimulated in vivo by lipoprotein lipase or by surface-active agents and may lead to transfer of apoproteins from VLDL to other lipoproteins.

REFERENCES

1. A. D. Dergunov, L. P. Anishkovich, and V. V. Shuvaev, Byull. Éksp. Biol. Med., **110**, 42 (1990).
2. A. D. Dergunov, V. V. Shuvaev, and N. V. Perova, Biokhimiya, **55**, 134 (1990).
3. Yu. M. Lopukhin, A. I. Archakov, Yu. A. Vladimirov, and E. M. Kogan, Cholesterolosis [in Russian], Moscow (1983).
4. Affinity Chromatography: Principles and Methods, Uppsala (1978).
5. S. J. Barr, B. A. Kottke, J. Y. Chang, and S. J. T. Mao, Biochim. Biophys. Acta, **663**, 491 (1981).
6. A. R. Clarke and J. J. Holbrook, Biochim. Biophys. Acta, **827**, 358 (1985).
7. P. N. Herbert, R. S. Shulman, R. I. Levy, and D. S. Fredrichson, J. Biol. Chem., **248**, 4941 (1973).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., **193**, 265 (1951).
9. S. J. T. Mao, P. K. Bhatnagar, A. M. Gotto, Jr., and J. T. Sparrow, Biochemistry, **19**, 315 (1980).
10. S. J. T. Mao, J. P. Miller, A. M. Gotto, Jr., and J. T. Sparrow, J. Biol. Chem., **255**, 3448 (1980).
11. T. G. Milner, K. W. S. Ko, T. Ohnishi, and S. Yokoyama, Biochim. Biophys. Acta, **1082**, 71 (1991).
12. G. Scatchard, Ann. New York Acad. Sci., **51**, 660 (1949).
13. M. Steiner, Biochim. Biophys. Acta, **805**, 53 (1984).
14. M. J. Tikkanen, R. Dargar, B. Pfleger, et al., J. Lipid Res., **23**, 1032 (1982).