ELIMINATION OF LOW-DENSITY LIPOPROTEIN-POLYANION INTERACTION BY AMINO MODIFICATIONS

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1. Introduction

Low-density lipoprotein (LDL) is selectively precipitated from human serum at physiological pH by macromolecular polyanions. Several studies on the mechanism of this interaction have been carried out [1–6], and it appears that it is an electrostatic phenomenon. To test this hypothesis further we changed the charge profile of the native LDL molecule by chemical modification of its free amino groups. LDL was acylated with succinic, maleic and acetic anhydrides and ethyl thioltrifluoroacetate, and the interaction of the modified LDL with three polyanions was studied over a wide range of polyanion/LDL ratios. None of these modified lipoproteins would precipitate with the polyanions amylopectin sulfate (APS), dextran sulfate (DS) or heparin (P).

2. Materials and methods

Succinic, maleic, and acetic anhydrides of the highest purity available commercially were used without further purification. Ethylthioltrifluoroacetate was prepared by refluxing ethanethiol and trifluoracetic anhydrydride for 8 hr and then collecting the 84°–86°C boiling material by fractional distillation. This material was further purified by redistillation. APS was prepared in our laboratory by a method previously described [7]; DS was obtained from Nutritional Biochemicals and HP was from Ryker Laboratories with a potency of 124 units/mg.

LDL was isolated from fresh pooled human serum either by a combination of APS precipitation and ultracentrifugation or by ultracentrifugation alone between the densities 1.006 and 1.063 g/ml [8]. The isolated LDL, in 0.05 M sodium phosphate buffer, pH 7.4, 0.01% in EDTA, was stored until used under nitrogen at 0°C without freezing. Under these conditions LDL preparations remain free of turbidity for several months.

The acylations were carried out in 0.05 M sodium phosphate, 5 mg LDL protein/ml, using a 20 molar excess of reagent as calculated from the lysine content of LDL [7]. The pH of the reaction mixture was maintained between 7.5 and 8.0 by the addition of NaOH. In the trifluoroacetylation reaction the pH was maintained at 10. When there was no further pH change, usually less than 1 hr, the mixture was dialyzed against phosphate buffer, pH 7.4. The succinylated, maleylated, acetylated and trifluoroacetylated LDL are referred to respectively as s-LDL, m-LDL, a-LDL, and tfa-LDL. The extent of LDL modification by acylation is greater than 90% [9, 10].

The precipitation of both native and modified LDL was studied over a polyanion/LDL ratio, w/w, in the range of 10^{-3} to 10^{2} in 0.05 M phosphate buffer, pH 7.4, 0.01% in EDTA. Each tube contained 0.5 mg LDL protein (LDL_p) and a variable amount of polyanion, the total volume being 4.0 ml. In the case of HP each tube contained 0.1 M $MgCl_2$. The absorbance was read at 680 nm after 15 min.

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3. Results and discussion

With APS the precipitation of native LDL varies with the APS/LDL_p ratio as seen in curve A, fig. 1, the maximum precipitation occurring at a 0.2–0.3 ratio. Essentially the same curve is found when DS is used as the precipitating polyanion. The same general shape of curve is demonstrated when LDL interacts with HP (curve A, fig. 2), there being a definite region of polyanion/LDL ratio (about 10) where precipitation is optimal. Thus, the APS-DS and HP curves are qualitatively similar but quantitatively different with respect to polyanion/LDL ratios necessary for optimum precipitation.

The positive charge of the amino group is replaced by the negative charge of a carboxyl group in the s-LDL and m-LDL. The amino-modified moieties of a-LDL and tfa-LDL possess no charge. The interaction of modified LDL with polyanions should be reduced in all cases if (*a*) this interaction is electrostatic in nature and (*b*) the free amino groups participate in the interaction. That amino groups are involved in the interaction is illustrated in curve B of both figs. 1 and 2. The precipitation of LDL by APS, DS and HP was completely eliminated for all the acylated molecules. Thus, strong supportive evidence is added to that body of data viewing the LDL-polyanion precipitation as a simple acid-base interaction.

There appears to be one major flaw in this acid-base concept, i.e. LDL is also an acidic molecule with an isoelectric point around pH 5.7. We have previously proposed [1] that there exists an uneven charge distribution between the outside surface of LDL and its interior which is inaccessible to macromolecular polyanions. Such an uneven charge characteristic resolves the objection to an acid-base type mechanism. This charge partition seems to be maintained largely by the secondary and tertiary structures of the protein moiety of LDL, because the precipitation with polyanions is also eliminated in the presence of 6M urea. Since the secondary strcuture of LDL is not altered by succinylation [10] it seems highly unlikely that the lack of precitability of s-LDL is due to a disruption of native protein conformation.

Although the acylations reported here eliminate the precipitation of LDL by polyanions, the possibility still exists that a soluble lipoprotein-polyanion complex is formed. To check this possibility s-LDL and APS were studied both together and separately by moving boundary electrophoresis and no soluble complexes could be found.

These data demonstrate that LDL-polyanion binding occurs through electrostatic bonding and that free amino groups play an essential role in this reaction.

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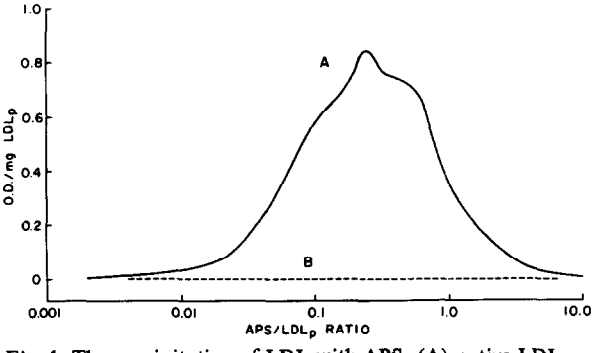

Fig. 1. The precipitation of LDL with APS: (A) native LDL, (B) modified LDL (s-LDL, m-LDL, a-LDL and tfa-LDL).

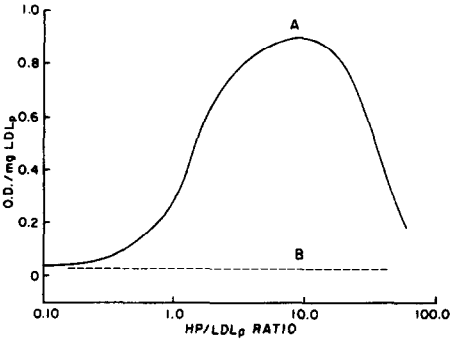

Fig. 2. The precipitation of LDL with HP: (A) native LDL, (B) modified LDL (s-LDL, m-LDL and tfa-LDL).

References

[1] R.S.Levy and C.E.Day, in: Proceedings Second International Symposium on Atherosclerosis (Springer, New York, 1970) in press.
[2] J.Canal and M.L.Girard, Bull. Soc. Chim. Biol. 50 (1968) 1523.
[3] M.L.Girard and J.Canal, Compt. Rend. Acad. Sci., Paris, Ser. D. 266 (1968) 842.
[4] M.Burnstein and R.Morfin, Rev. Franc. Etudes Clin. Biol. 13 (1968) 274.
[5] T.Nishida, J. Lipid Res. 9 (1968) 627.
[6] P.Bernfeld, J.S.Nisselbaum, B.J.Berkeley and R.W.Hanson, J. Biol. Chem. 235 (1960) 1852.
[7] R.S.Levy, A.C.Lynch, E.D.McGee and J.W.Mehl, J. Lipid Res. 8 (1967) 463.
[8] C.E.Day and R.S.Levy, J. Lipid Res. 9 (1968) 789.
[9] A.M.Gotto, R.I.Levy, M.E.Birnbaumer and D.S.Fredrickson, Nature 223 (1969) 835.
[10] A.Scanu, H.Pollard and W.Reader, J. Lipid Res. 9 (1968) 342.