PLASMA HIGH DENSITY LIPOPROTEINS INHIBIT THE ACTIVATION OF COAGULATION FACTOR X BY FACTOR VIIa AND TISSUE FACTOR

Steven D. CARSON
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510, USA

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1. Introduction

Activation of coagulation factor X by factor VIIa and tissue factor [1] presumably occurs in response to tissue damage. This was an important coagulation reaction to study with respect to plasma high density lipoprotein (HDL) because:

(1) Tissue factor is a membrane protein dependent on association with a phospholipid bilayer for its cofactor activity [2–4];
(2) HDL is capable of destabilizing phospholipid vesicles [5–8] and could therefore influence the initiation of coagulation by tissue factor;
(3) HDL is statistically elevated in populations with low incidence of atherosclerosis [9–14].

Investigations of lipoprotein effects on the intrinsic (contact) pathway of coagulation, and on activation of prothrombin, have shown that lipoproteins can serve as the phospholipid required in these reactions [15–17]. These studies, however, dealt with the final reactions of the pathway rather than the early reactions, or did not isolate the primary reactions from later lipid-dependent reactions. This report describes the inhibitory effect of HDL on the extrinsic pathway of coagulation using the isolated reaction, tissue factor-dependent activation of factor X.

2. Materials and methods

Lipoproteins from fasted donors were separated from plasma by ultracentrifugation [18] using KBr steps of 1.26 g/ml, 1.21 g/ml and 1.06 g/ml, overlayed with a step of 0.15 M NaCl. The 1.06 g/ml fraction and 1.21 g/ml fraction (IIDL) were collected, made to 1.26 g/ml with KBr, and reisolated by a second centrifugation. The isolated lipoproteins were dialyzed against 0.05 M Tris, 0.1 M NaCl, 0.01% NaN_3 (pH 7.6) (Tris–saline buffer). The HDL and 1.06 g/ml fractions were examined by immunoelectrophoresis [19] and by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) [20]. The lipid and protein components of HDL were separated by extraction twice with an equal volume of chloroform–methanol (2:1) [21]. The solvents were evaporated from the lipids under N_2. The apoproteins were dialyzed against Tris–saline buffer and stored at −20°C. The lipids were resuspended in Tris–saline buffer and sonicated under N_2 in a bath sonicator (Branson) just prior to use.

Human tissue factor was purified 2000-fold from placenta and reconstituted with bovine brain lipids by dialysis [3]. Bovine brain tissue factor was a homogeneous preparation kindly provided by Dr Ron Bach [22,23]. The bovine tissue factor was reconstituted with bovine brain lipids; $CdCl_2$ and deoxycholate were removed by gel filtration of the reconstituted mixture (1 ml containing 1.14 ng tissue factor, 0.5 mg mixed brain lipids in deoxycholate, 5 mM $CdCl_2$ and bovine serum albumin at 1 mg/ml [3,4]) on a column of Bio-Gel A5m (13.5 × 1.5 cm). Tissue factor coagulant activity was measured in a 2-stage assay [24] in which purified bovine factors X and VIIa (provided by Dr Yale Nemerson) were used in the first stage at 19.8 and 0.5 nM, respectively.

The radiometric assay in [25] was used to measure the activation of factor X by factor VIIa and tissue factor. The assay concentrations of tritiated factor X (148 000 cpm/μg) and factor VIIa were 422 nM and 1.49 nM, respectively. The effects of lipoproteins on tissue factor activity were assessed by incubating the lipoproteins with relipidated tissue factor at 37°C in 160 μl. The remaining reagents were added after 15 min and gave a final reaction volume of 200 μl.

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Lipoprotein effects on tissue factor coagulant activity were assessed by combining relipidated tissue factor and purified lipoprotein, (final vol. 100 μl) in microtiter wells. The mixture was incubated at 37°C. After 15 min, 20 μl of the tissue factor lipoprotein mixture were combined with factor X, VIIa, and Ca^{2+} in the first stage of the coagulation assay (first stage volume was 60 μl).

Protein concentrations were determined by amino acid analysis on a Beckman 121M analyzer after 24 h acid hydrolysis. Phospholipid concentrations were determined according to [26].

3. Results

The experiments with human placental tissue factor showed that HDL inhibited tissue factor coagulant activity (fig.1). The ability of the protein constituents of HDL (apoHDL) to withstand organic extraction of the HDL lipids permitted separate evaluation of apoHDL and HDL lipids. Chloroform–methanol extraction reduced the lipid/protein ratio from ~1 nmol PO_4/μg HDL protein in the intact lipoprotein, to <0.1 nmol PO_4/μg protein in the apoHDL preparations. The

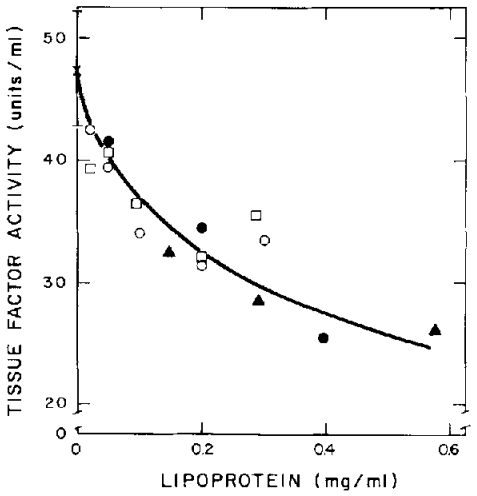

Fig.1. Effects of HDL (open symbols) and apoHDL (closed symbols) on human placental tissue factor. Lipoprotein concentrations are those used in the 0.1 ml incubation with tissue factor. Donors were G.G. (○,●) and S.D.C.1 (□). One apoHDL was from HDL kindly provided by Dr Janet Boyles from an anonymous donor (▲). The phospholipid content of the preparations (nmol PO_4/μg protein) were: 1.0 (○); 0.7 (□); <0.02 (●); 0.09 (▲).

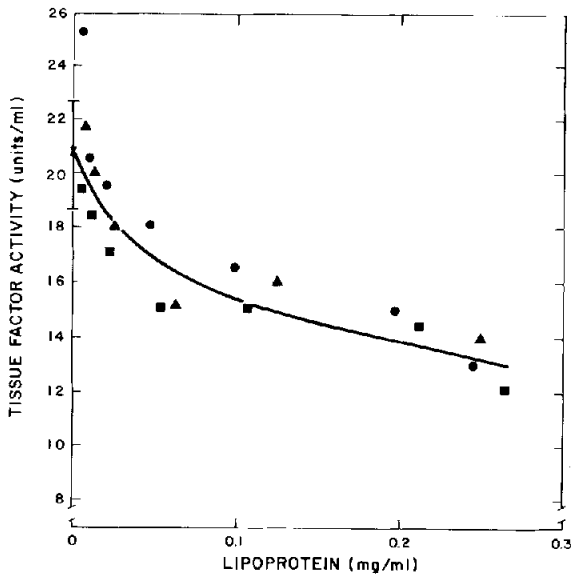

Fig.2. Effects of HDL on bovine brain tissue factor coagulant activity. Lipoproteins were prepared from donors S.D.C.2 (●), J.S. (▲) and S.M.C. (■).

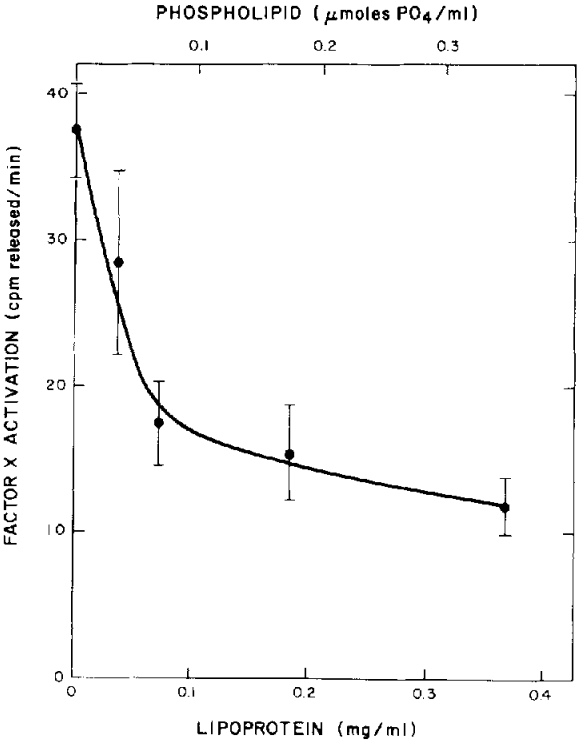

Fig.3. Effect of HDL on the radiometric assay of factor X activation by tissue factor and factor VIIa. The plasma donor was S.D.C. Lipoprotein concentrations are those in the complete reaction mixture (200 μl).

Table 1
ApoHDL and HDL lipid effects on bovine brain tissue factor coagulant activity (tissue factor alone: 20.7 ± 2.0 units/ml)

Donor: S.D.C. 2			Donor: S.M.C.		
apoHDL[a] (μg)	Lipid[a] (nmol PO_4)	Tissue factor (units/ml)	apoHDL (μg)	Lipid (nmol PO_4)	Tissue factor (units/ml)
2.0		13.0	2.0		15.5
5.0	<1.6	13.5	5.0	0	13.0
10.0		8.8	10.0		12.5
	[b]		–	4.0	20.0
			–	8.0	22.0
			–	20.0	24.0
			–	40.0	23.5

[a] Quantities are as used in the 0.1 ml incubation with tissue factor; concentrations (μg/ml) used in the first stage of the assay are derived by multiplying by 3.33

[b] This chloroform–methanol extract of HDL inhibited tissue factor activity, but the electrophoretic analysis revealed a prominent apoA-I protein. All other HDL lipid preparations contained no detectable protein component

apoHDL, as for the intact lipoprotein, inhibited tissue factor activity (fig.1). In the presence of the extracted lipids (tested at 46–260 nmol PO_4/ml), however, full tissue factor activity was retained (42.6–52.0 units/ml).

The results obtained with human placental tissue factor were confirmed using a homogeneous preparation of bovine brain tissue factor. HDL from 3 donors inhibited tissue factor coagulant activity (fig.2). ApoHDL inhibited bovine tissue factor coagulant activity (table 1) while the lipid component was not inhibitory. Conclusive evidence that the inhibition of coagulant activity was due to lipoprotein effects on the activation of factor X by tissue factor and factor VIIa was obtained using the direct radiometric assay of factor X activation. HDL produced a striking decrease in the velocity of factor X activation (fig.3).

The 1.06 g/ml preparations also inhibited tissue factor coagulant activity. Although these preparations had each produced a single immunoprecipitate similar to LDL, small amounts of protein other than apoB were detected after electrophoresis in the presence of SDS. This indicated that the 1.06 g/ml fraction probably contained some VLDL or IDL in addition to LDL. Studies with the 1.06 g/ml preparations were not pursued further.

4. Discussion

Based on 1.2 mg apoA-I/ml, the major protein component of HDL [27–29], HDL at 8.3% of plasma concentration inhibited coagulant activity of both human and bovine tissue factor by ~25%. In the radiometric assay, the same concentration of HDL inhibited factor X activation by 54%. In the coagulation assay, factors X and VII are used at 25% and 6.5% of their respective human plasma concentrations. They are used at 520% (factor X) and 20% (factor VII) of their plasma concentrations in the radiometric assay [30,31]. Therefore, in all of the experiments, inhibition of factor X activation was observed when the concentrations of HDL relative to factors X and VII were near or below their respective levels in human plasma.

Although phospholipid constitutes 25% or more of the weight of holoHDL [32], extracted lipids had no inhibitory effect on tissue factor coagulant activity. On the other hand, apoHDL was as inhibitory as holoHDL. These results suggest that inhibition of tissue factor activity is due to a specific interaction between the lipoprotein and tissue factor. The principal protein of HDL, apo-A-I, is capable of exchanging from HDL to phospholipid vesicles [5–8]. ApoA-II, apoC, and apoE are also reversibly associated with plasma lipoproteins [33]. Exchange of apolipoproteins from lipoproteins to vesicles containing tissue factor could alter the tissue factor coagulant activity by direct effect on tissue factor, by restricting the access of enzyme and substrate to the cofactor–

vesicle surface, or by altering some essential structural feature of the vesicle. The presence of tissue factor in arterial endothelium and atherosclerotic plaques [34], the ability of lipoproteins to inhibit tissue factor-dependent activation of factor X while promoting the factor Xa conversion of prothrombin to thrombin [15–17], and the relationship between plasma lipoproteins and cardiovascular disease [9–14] suggest that understanding tissue factor expression and regulation may be relevant to studies of atherosclerosis.

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References

[1] Nemerson, Y. (1966) Biochemistry 5, 601–608.
[2] Carson, S. D. and Konigsberg, W. H. (1980) Science 208, 307–309.
[3] Carson, S. D. and Konigsberg, W. H. (1980) Thrombos. Haemostas. 44, 12–15.
[4] Carson, S. D. and Konigsberg, W. H. (1981) Biosci. Rep. 1, 197–205.
[5] Tall, A. R. and Small, D. M. (1977) Nature 265, 163–164.
[6] Scherphof, G., Roerdink, F., Waite, M. and Parks, J. (1978) Biochim. Biophys. Acta 542, 296–307.
[7] Tall, A. R., Hogan, V., Askinazi, L. and Small, D. M. (1978) Biochemistry 17, 322–326.
[8] Chobanian, J. V., Tall, A. R. and Brecher, P. I. (1979) Biochemistry 18, 180–187.
[9] Barr, D. P., Russ, E. M. and Eder, H. A. (1951) Am. J. Med. 11, 480–493.
[10] Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. and Dawber, T. R. (1977) Am. J. Med. 62, 707–713.
[11] Miller, G. J. and Miller, N. E. (1975) Lancet i, 16–19.
[12] Rhoads, G. G., Gulbrandsen, C. L. and Kagan, A. (1976) New Eng. J. Med. 294, 293–298.
[13] Riesen, W. F., Mordasini, R., Salzmann, C., Theler, A. and Gurtner, H. P. (1980) Atherosclerosis 37, 157–162.
[14] Hamada, N. (1980) Jap. Circ. J. 44, 487–494.
[15] Vijayagopal, P. and Ardle, N. G. (1978) Thrombos. Res. 12, 721–733.
[16] Bajaj, S. P., Harmony, J. A. K., Martinez-Carrion, M. and Castillino, F. J. (1976) J. Biol. Chem. 251, 5233–5236.
[17] Sandberg, H. and Andersson, L.-O. (1976) Thrombos. Haemostas. 35, 178–185.
[18] Havel, R. J., Eder, H. A. and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345–1353.
[19] Grabar, P. and Williams, C. A. jr (1953) Biochim. Biophys. Acta 10, 193–194.
[20] Laemmli, U. K. (1970) Nature 227, 680–685.
[21] Folch, J., Ascoli, I., Lees, M., Meath, J. A. and LeBaron, F. N. (1951) J. Biol. Chem. 191, 833–841.
[22] Bach, R., Nemerson, Y. and Konigsberg, W. H. (1981) J. Biol. Chem. in press.
[23] Nemerson, Y., Zur, M., Bach, R. and Gentry, R. (1980) in: Regulation of Coagulation (Mann, K. G. and Taylor, F. B. eds) pp. 193–202, Elsevier/North-Holland, Amsterdam, New York.
[24] Pitlick, F. A. and Nemerson, Y. (1976) Methods Enzymol. 45, 37–48.
[25] Silverberg, S. A., Nemerson, Y. and Zur, M. (1977) J. Biol. Chem. 252, 8481–8488.
[26] Bartlett, G. R. (1959) J. Biol. Chem. 234, 466–469.
[27] Albers, J. J., Cheung, M. C. and Wahl, P. W. (1980) J. Lipid Res. 21, 874–878.
[28] Miller, J. P., Mao, S. J. T., Patsch, J. R. and Gotto, A. M. jr (1980) J. Lipid Res. 21, 775–780.
[29] Lopes-Virella, M. F. L., Virella, G., Evans, G., Malenkos, S. B. and Colwell, J. A. (1980) Clin. Chem. 26, 1205–1208.
[30] Miletich, J. P., Jackson, C. M. and Majerus, P. W. (1978) J. Biol. Chem. 253, 6908–6916.
[31] Broze, G. J. and Majerus, P. W. (1980) J. Biol. Chem. 255, 1242–1247.
[32] Tanford, C. (1980) in: The Hydrophobic Effect: Formation of Micelles and Biological Membranes, pp. 165–180, Wiley, London, New York.
[33] Osborne, J. C. and Brewer, H. B. jr (1980) Ann. NY Acad. Sci. 348, 104–112.
[34] Zeldis, S. M., Nemerson, Y., Pitlick, F. A. and Lentz, T. L. (1972) Science 175, 766–768.