Vol. 216, No. 1, 1995 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 2, 1995 Pages 414-421

NONCOVALENT INTERACTIONS IN MAINTAINING THE NATIVE STRUCTURE OF LOW DENSITY LIPOPROTEIN

Susan Lakatos¶, József Fűrész, Éva Pállinger, Katalin Rischák*, Katalin Schweitzer and Lajos Szollár*

Department of Pathophysiology, Research Centre of Military Medicine, Hung.Army, Budapest, P.O.Box 19, H-1456, Hungary

*Institute of Pathophysiology, Semmelweis University School of Medicine, Budapest, Hungary

Received October 2, 1995

The integrity and the surface charge distribution of native low density lipoprotein (LDL) are prerequisites of its binding to the LDL receptor. Oxidation is one of the most important physiological effects resulting in an altered structure and metabolism of LDL. To reveal forces responsible for maintaining the intact structure of LDL in the absence of cells we have determined the kinetics of lipid peroxidation, changes in electrophoretic mobilities and size distributions of LDL samples as a function of Cu^{++} induced oxidative modification in a cell-free system at two different (50 mM and 150 mM) ionic strengths by electrophoretic and dynamic light scattering. Our data show that the lipid peroxidation is almost complete before LDL is degraded at 50 mM while a slight extent of lipid peroxidation is enough to result in the same effect at 150 mM. These suggest that both ionic and hydrophobic interactions are necessary to maintain the integrity of the LDL molecule.

The elevated level of circulating cholesterol is one of the most important risk factors for atherosclerosis. Cholesterol is carried in the plasma mainly by the low density lipoprotein (LDL). In the last decade a series of remarkable studies (1-4) has demonstrated that oxidation of LDL is an important in vivo phenomenon with potential to explain the chain of events leading to the development of atherosclerosis. Several methods are used to monitor in vitro oxidation of LDL. The parameters measured are mainly correlated with lipid peroxidation: e.g. thiobarbituric acid reactive substances (TBARS), conjugated dienes, aldehydes, etc. Only a few attempts have been made to describe time course of changes in the whole LDL molecule as a consequence of oxidation (2,5,6), and in most cases the fully oxidized state has been compared to the native one. Increased content of malondialdehyde (MDA) and conjugated dienes, increased fluorescence, electrophoretic mobility and tendency to aggregate, degradation of the

¶ *Corresponding author. FAX: (361)-140-1144.*

0006-291X/95 $12.00

apoprotein part of the molecule have been reported to be the main chemical and physico-chemical features of oxidized LDL (cf. reviews of Esterbauer et al. (3) and Steinberg (4) and refs. herein).

LDL is an oily droplet containing a core of hydrophobic cholesterol esters and a monolayer of phospholipids and free cholesterol covering about 70% of the surface. The remaining 30% of the surface is covered by a huge apoprotein (apoB) stabilizing the particle in the aqueous phase of the blood (7-9). Mapping apoB by immunoelectron microscopy showed its extension over at least a hemisphere of the LDL surface (10). Based on a detailed analytical ultracentrifugation study of LDLs containing C-terminally truncated apoB fragments, Spring et al. (9) suggested that apoB penetrated the surface monolayer to make direct physical contact with the hydrophobic lipid core to complement phospholipids and cholesterols.

In light of these findings the aims of our study were (i) to identify non-covalent interactions responsible for maintaining the integrity of LDL particle as a whole and (ii) to contribute to the physico-chemical characterization of changes occurring during in vitro oxidation of LDL in the absence of cells.

MATERIALS AND METHODS

Isolation of LDL

LDL was isolated by preparative ultracentrifugation from freshly collected normal human plasma containing anticoagulant. Plasma was adjusted to a density of 1.4 g/ml by addition of NaBr. 10 ml of 1.4 g/ml plasma was centrifuged in a VTi 50 rotor (Beckman Instr. Inc., California, USA) at 45,000 RPM for 3.5h at 4 °C in a density gradient formed from 5 ml 1.3 g/ml NaBr and 24 ml 1.006 g/ml NaCl solutions. After centrifugation the LDL samples were dialyzed against either 50 mM phosphate buffer, pH 7.4 or 50 mM phosphate buffer containing 100 mM NaCl, pH 7.4, for 36 hours with four changes of buffer.

Modification of LDL

After dialysis the LDL was filtered through a 0.22 μm pore-size filter to remove insoluble aggregates formed during the dialysis and to sterilize the solution. The protein concentration was measured by Lowry method with BSA as standard (11). Oxidation was initiated in 0.8 mg/ml solutions of LDL by addition of 3 μM $CuCl_2$ and followed by incubation at 37 °C for various period of time. Control samples were kept either at 5 or 37 °C, without $CuCl_2$.

Characterization of LDL

Native and modified forms of LDL were characterized by the following procedures:

1. Measurement of electrophoretic mobility and particle size distribution of modified and native LDL was carried out by electrophoretic and dynamic light scattering with an Photal Otsuka Electronics ELS 800 apparatus. In electrophoretic light scattering experiments the

Doppler shift of the incident light was detected by heterodyne method and analyzed as described by Oka et al. (12). using the software provided by Otsuka Electronics. In dynamic light scattering experiments the autocorrelation functions obtained by High Speed Full Correlator Unit (Photal Otsuka Electronics, Japan) were analyzed by cumulant (13) and histogram methods. The intensity-, weight- and number-averages of Stokes' radii were calculated from the respective diffusion coefficients. Data were processed on a Compaq Deskpro 286e computer using the software provided by Photal Otsuka Electronics, Japan.

2. Concentrations of thiobarbituric acid reactive substances (TBARS) in LDL samples were determined according to Placer et al. (14).

RESULTS AND DISCUSSION

Altogether 11 samples were analyzed and compared at two different ionic strengths. Fig. 1A and 1B show the results of electrophoretic light scattering experiments measured at 50 mM and at 150 mM ionic strength respectively. In accordance with the theoretical calculations (16) native LDL has higher electrophoretic mobility at 50mM [μ_o=(-1.45±0.20)*10^{-4} cm^2/Vsec] than at 150 mM ionic strength [μ_o=(-0.42±0.12)*10^{-4} cm^2/Vsec]. The relative electrophoretic mobility of samples (oxidized/native) - after a transient decrease - increased at both ionic strengths as a function of incubation time showing that during oxidation the net negative surface charge of LDL particle is increasing (for review see (17)). This increase is slightly faster at 150 mM than at 50 mM in the first 10 hours.

The transient decrease in electrophoretic mobility at the beginning of oxidation can be explained by the introduction of positive charges into the system by addition of Cu^{++}. The relatively slow compensation of those positive charges due to the oxidative process (Fig. 1A and 1B), supports that oxidation products may attack (18) and/or convert certain amino acids like His to Asp and Pro to Glu as suggested by Stadtman (19).

Histogram analysis of dynamic light scattering data provided diameter values of native LDL from different healthy donors. A significant difference could be detected in the weight-average Stokes' diameter (d_o) of native LDL using buffers of different ionic strength: at 50 mM d_o=16.5±0.1 nm while at 150 mM do=18.6±0.4 nm. At physiological ionic strength this method resulted in somewhat lower values than those generally accepted in the literature (20-27 nm; (20-22)). Since apoB is a glycoprotein (23) and either gel electrophoresis or density gradient centrifugation have been mostly used to measure the size of LDL or apoB (20-23), those values can be considered as upper limits. However, our data are consistent with those measured by small angle X-ray scattering (24) which method likewise dynamic light scattering provides information on the solvated molecule. The smaller hydrodynamic diameter measured at 50 mM ionic strength indicates that at physiological ionic strength (150 mM) the LDL molecule has a less compact structure than at low (50 mM) ionic strength.

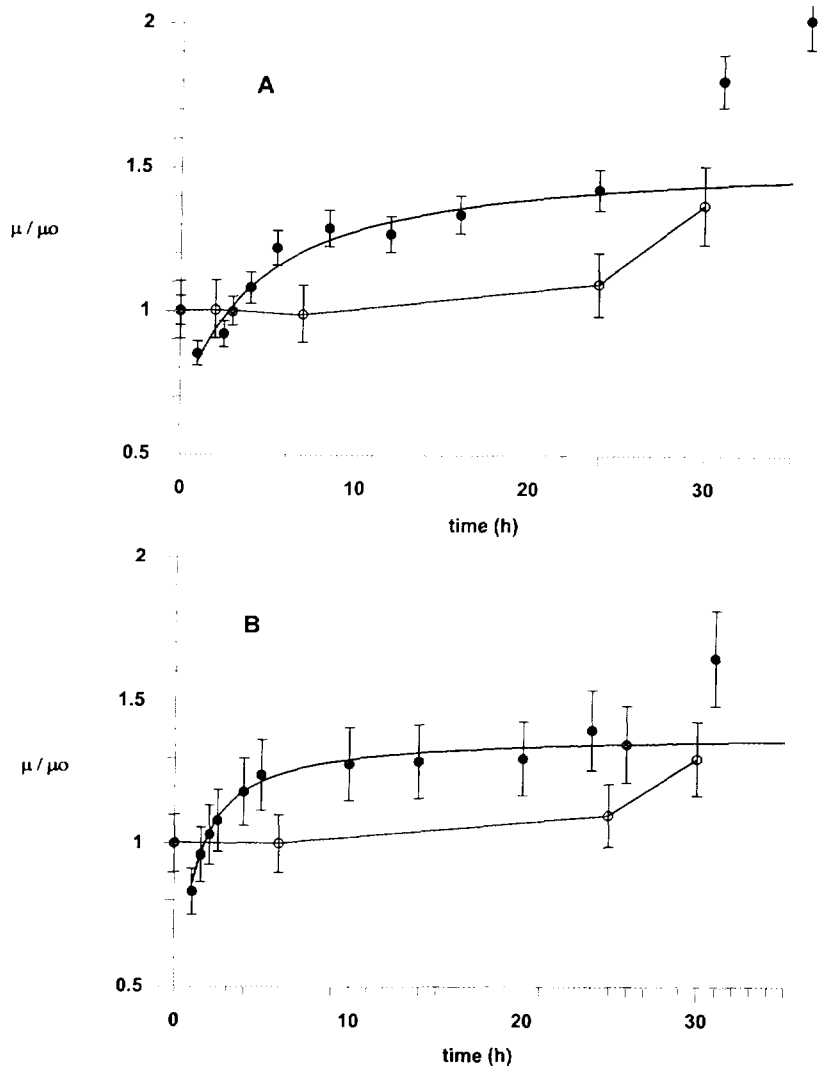

Fig. 1. Kinetics of relative electrophoretic mobilities of LDL oxidized by Cu^{++} at 50 mM (A) and at 150 mM (B) ionic strength. μ_o is the mobility of the sample measured before addition of Cu^{++}. The open symbols refer to control sample kept at 5 °C without Cu^{++}.

The kinetics of normalized average LDL particle size upon oxidation at 50 mM and 150 mM ionic strengths obtained by cumulant method are shown in Fig. 2A and 2B respectively. The time course of the increase of the average size is slightly faster at 150 mM than at 50 mM in the first 10 hours.

Histogram analysis of the same data provided a more detailed picture: the monotonous increase in the relative diameter obtained by the cumulant method (13) is a resultant of two different processes: formation of particles smaller (0.5nm<d<7nm) than the native ones and

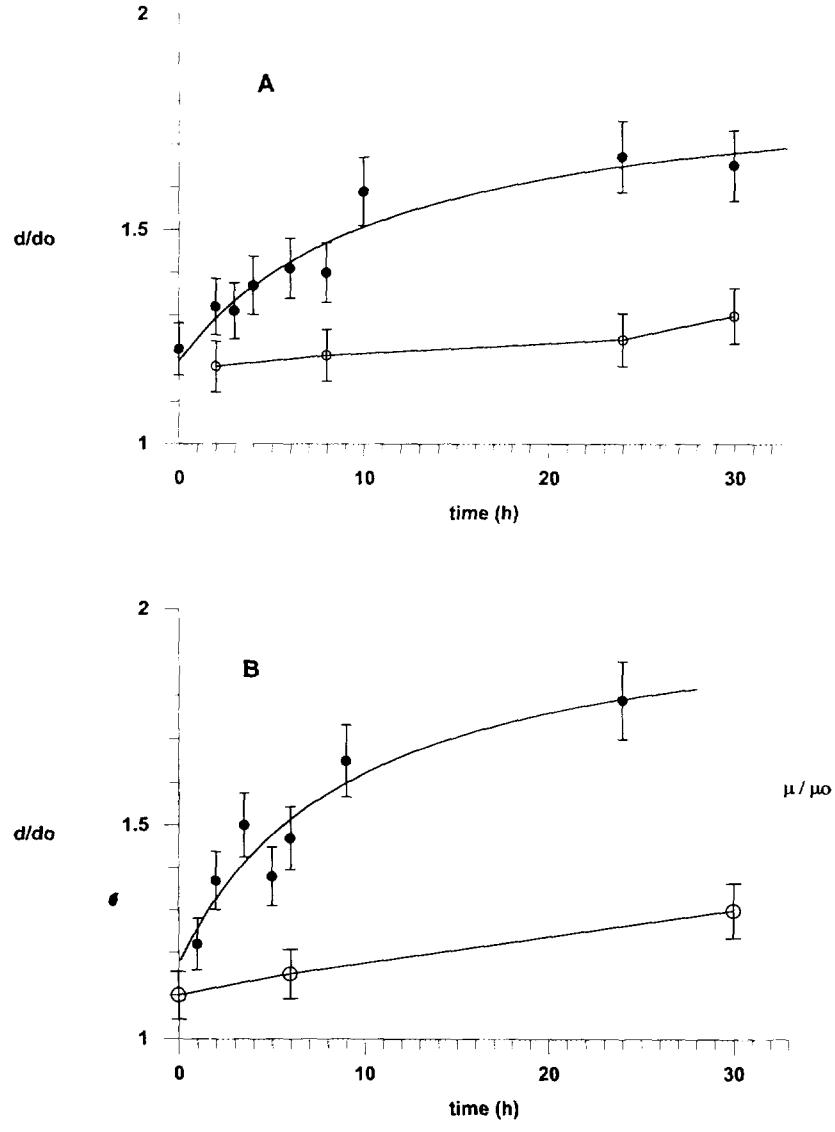

Fig. 2. Kinetics of normalized average hydrodynamic diameter of LDL oxidized by Cu^{++} at 50 mM (A) and at 150 mM (B) calculated by cumulant method . d_0 is the weight average hydrodynamic diameter of the native molecule. Open symbols refer to control, filled symbols refer to oxidized samples.

aggregation. At 50 mM ionic strength the small-sized fraction appears later (4-6 hours) than at 150 mM (0-3 hours).

Aggregates of LDL with higher diameter could be formed in several different ways: either from small-sized fractions or from truncated LDL molecules, or from any variations of the above components. During oxidation apoB is degraded (6), and some of its fragments might dissociate from the molecule (taking some lipid or not) and leave behind unmasked apolar

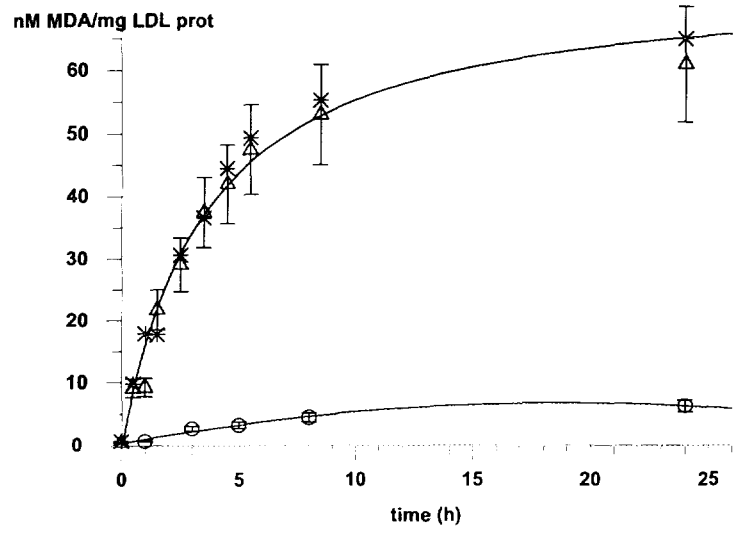

Fig. 3. Kinetics of Cu^{++} stimulated oxidation of LDL measured by change of TBARS at 50 mM (*-*) or at 150 mM (Δ-Δ) ionic strength; o-o , control sample incubated at 5 °C without Cu^{++}.

patch(es) on the surface. These latter forms of LDL are possible candidates to form huge aggregates in LDL samples.

The tendency of aggregation as a function of degree of oxidation was the same for all the preparations investigated by us, but aggregates appeared later at 50 mM than at 150 mM ionic strength. However, we have to admit that the time when aggregates became detectable varied from prep to prep both at physiological (150mM) and at low (50mM) ionic strengths, probably because of different antioxidant levels of sera obtained from various donors and because of individual differences in susceptibility to oxidize LDL (3,4,17,25).

The time course of lipid peroxidation as measured by TBARS (Fig. 3) concentrations does not depend on ionic strength of the buffer. Fig. 3 shows that the extent of lipid peroxidation is about 90% at the 5th hour and can be considered as complete at the 10th hour after addition of Cu^{++}. The concentration of TBARS does not change in the first 20 hours in control samples stored at 5 °C without Cu^{++}. The lack of well known lag-phase (3,17) in the time course of formation of TBARS shows that our samples were already oxidized to a slight extent at the beginning of the experiments, i.e. at the addition of $CuCl_2$. Taken altogether, the ionic strength independence of TBARS reactivity of our samples and the ionic strength dependence of formation of small-sized fraction suggest that at 50 mM ionic strength the lipid peroxidation is almost complete before LDL is degraded, while at 150 mM ionic strength a slight extent of

lipid peroxidation is enough to result in the same effect. Thus, one can conclude, that both ionic and hydrophobic forces are necessary to maintain the native structure of LDL particle.

ACKNOWLEDGMENTS

The skilful technical assistance of Ms. Zs. Szabados is greatly acknowledged. Thanks are due to Prof. J.Hideg for his continuous interest and encouragement and to Dr. W.Otani (Photal Otsuka Electronics, Japan) for his continuous technical support and discussion during the experiments.

REFERENCES

1. Steinberg, D., Parthasarathy, S., Carew, T., Khoo, J.C. and Witztum, J.I. (1989) N. Engl. J. Med. 320, 915-924
2. Esterbauer, H., Dieber-Rotheneder, M., Waeg, G., Striegl, G. and Jürgens, G. (1990) Chem. Res. Toxicol. 3, 77-92
3. Esterbauer, H., Gebicki, J., Puhl, H. and Jürgens, G. (1992) Free Rad. Biol. Med. 13, 341-390
4. Steinberg, D. (1993) J. Int. Med. 233, 227-232
5. Esterbauer, H., Waeg, G., Puhl, H., Dieber-Rotheneder, M. and Tatzber, F. *Inhibition of LDL Oxidation by Antioxidants in Free Radicals and Aging*, Basel, Switzerland: Birkhauser Verlag, 1992. pp. 145-157
6. Hoff, H.F., Whitaker, T.E. and O'Neil, J. (1992) J. Biol. Chem. 267, 602-609
7. Atkinson, D., Small, D.M. and Shipley, G.G (1980) Ann. N. Y. Acad. Sci. 348, 284-298
8. Laggner, P., Kostner, G.M., Rakusch, U. and Worcester, D.(1981) J. Biol. Chem. 256, 11832-11839
9. Spring, D.J., Chen-Liu, L.W., Chatterton, J.E., Elovson, J. and Schumaker, V.N. (1992) J. Biol. Chem. 267, 14839-14845
10. Chatterton, J.E., Phillips, M.L., Curtiss, L.K., Milne, R.W., Marcel, Y.L. and Schumaker, V.N. (1991) J. Biol. Chem. 266, 5955-5962
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275
12. Oka, K., Otani, W., Kameyama, K., Kidai, M. and Takagi, T. (1990) Appl. Theor. Electrophor. 1, 273-278
13. Koppel, D.E. (1972) J. Chem. Phys. 57, 4814-4820
14. Placer, Z.A., Cushman, L. and Johnson, B.C. (1966) Anal. Biochem. 16, 359-364
15. Esterbauer, H., Striege, G., Puhl, H. and Rothender, M. (1989) Free Rad. Res. Commun. 6, 67-75
16. Miklavic, S.J. and Ninham, B.W. (1989) J. theor. Biol. 137, 71-89
17. Gebicki, J.M., Jürgens, G. and Esterbauer, H. (1991) Oxidative Stress: Oxidants and Antioxidants pp. 371-397 Academic Press Ltd.
18. Jürgens, G., Lang, J. and Esterbauer, H. (1986) Biochim. Biophys. Acta 875, 103-114

19.Stadtman, E.R. (1989) Medical, Biochemical and Chemical Aspects of Free Radicals O. Hayaishi, E. Niki, M. Kondo, T. Yoshikawa, Eds. pp. 11-19, Elsevier Science Publishers B.V., Amsterdam

20.Krauss, R.M. and Burke, D.J. (1982) J. Lipid Res. 23, 97-104

21.Williams, P.T., Krauss, R.M., Nichols, A.V., Vranizan, K.M. and Wood, P.D.S. (1990) J. Lipid Res. 31, 1131-1139

22.Williams, P.T., Vranizan, K.M. and Krauss, R.M (1992) J. Lipid Res. 33, 765-774

23.Yang, C.Y., Gu, Z.W., Weng, S.A., Kim, T.W., Chen, S.H., Pownall, H.J., Sharp, P.M., Liu, S.W., Li, W.H., Gotto, A.M.,Jr. and Chan, L. (1989) Arteriosclerosis 9, 96-108

24.Baumstark, M.W., Kreutz, W., Berg, A., Frey, I. and Keul, J. (1990) Biochim. Biophys. Acta 1037, 48-57

25. Jialal, I., Vega, G.L. and Grundy, S.M. (1990) Atherosclerosis 82, 185-191